European Journal of Clinical and Experimental Medicine

e-ISSN 2544-1361
ISSN 2544-2406

Formerly: Medical Review

Quarterly

Vol. 17, No. 2
Publication date: June 2019

Rzeszów, Poland 2019

Technical development, layout and interior design: Wojciech Pączek
Cover design: Wiesław Grzegorczyk

ICV 2016 = 83.64
MNiSW: 7.0

Indexing:
Ministry of Science and Higher Education (Poland)
Index Copernicus
The Central EuropeanJournal of SocialSciences and Humanities (CEJSH)
POL-Index
Central Medical Library (Poland)
SPORT Computer Base
ARIANTA – Science and branch Polish electronic journals
J-Gate

e-ISSN 2544-1361
ISSN 2544-2406

EDITORIAL CORRESPONDENCE
European Journal of Clinical and Experimental Medicine Editorial Office
35-959 Rzeszów, ul. Kopisto 2A,
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http://www.ejcem.ur.edu.pl
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https://mc04.manuscriptcentral.com/pmur

PUBLISHER: PUBLISHING OFFICE OF THE UNIVERSITY OF RZESZÓW
35-959 Rzeszów, ul. prof. S. Pigonia 6,
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ISSN 2544-1361 (online); ISSN 2544-2406

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 112

Contents

© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.1

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 113–117

ORIGINAL PAPER

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Evaluation of platelet indexes as potential biomarkers of suspected pulmonary embolism

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ABSTRACT

Introduction. Pulmonary embolism is one of the most frequent cardiovascular diseases, potentially leading to death. There is no validated biomarker with both high specificity and sensitivity.

Aim. The aim of the study was to define the diagnostic importance of platelet count (PLT), mean platelet volume (MPV) and platelet distribution width (PDW) on acute pulmonary embolism.

Material and methods. We retrospectively reviewed the medical records of 145 patients with clinically suspected acute pulmonary embolism admitted to the Emergency Department. Demographic data and laboratory tests were collected on admission. All patients underwent computed tomography (CT) angiography.

Results. The total data of 145 patients were analyzed, including 65 patients (67±17 years; 30 men/35 women) with acute pulmonary embolism confirmed with CT and 80 patients (67±19 years; 26 men/54 women) with negative CT. The MPV did not differ between the patients with acute PE and the control group (8.0 fL [IQR: 7.6-8.4] vs. 7.9 fL [IQR: 7.4-8.7], p=0.45). There were no significant differences in PLT ($220x10^3/mm^3$ [IQR: 172-274] vs. $243x10^3/mm^3$ [IQR: 186-286], p=0.12) and PDW (59.0 ± 6.9% vs. 57.2 ± 7.3%, p=0.12).

Conclusions. Our results suggest that platelet indexes (at a single time point) are not a reliable diagnostic biomarkers of acute pulmonary embolism.

Keywords. mean platelet volume, platelet count, platelet distribution width, pulmonary embolism

Introduction

Pulmonary embolism (PE) and deep vein thrombosis (DVT) are 2 manifestations of venous thromboembolism (VTE).[1] It is one of the most frequent cardiovascular diseases with an overall annual incidence of 110-130 per 100,000 inhabitants.[1] In most cases, PE appears as a consequence of DVT and it may be lethal, lead to chronic disease or it can remain asymptomatic.[2]

Computed tomography (CT) angiography is the method of choice for diagnosing the patients with suspected PE but it is costly and not available 24 hours a day, 7 days a week in every hospital. Moreover, it is associated with contrast-induced nephropathy and with

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 6.02.2019 | Accepted: 6.05.2019
Publication date: June 2019

Wójcik M, Daszyk-Wójcik J, Skoczyński K. *Evaluation of platelet indexes as potential biomarkers of suspected pulmonary embolism.* Eur J Clin Exp Med. 2019;17(2):113–117. doi: 10.15584/ejcem.2019.2.1

radiation exposure.[3,4] D-dimer testing is sensitive but not specific for PE.[5] There is a need to disclose a reliable, noninvasive test that can precisely identify patients with PE.

Mean platelet volume (MPV) is the most commonly used measure of platelet size and a potential marker of platelet reactivity and inflammation.[6] Platelet distribution width (PDW) represents variation in platelet size.[7]

Platelets play a significant role in the pathogenesis of atherosclerosis. MPV is increased in acute coronary syndrome, mortality following myocardial infarct, restenosis following coronary angioplasty and stroke.[8-10] It was found that elevated MPV and PDW are also associated with an early phase of cerebral venous sinus thrombosis.[11] Recent pathophysiological studies indicated that etiopathogenetic mechanism in both venous and arterial thrombogenesis are similar.[12] Patients with cardiovascular risk factors for atherosclerosis have increased risk and severity of unprovoked VTE.[13]

To the present date, no validated biomarkers with both high specificity and sensitivity have been established for acute pulmonary embolism (APE).

Aim

The aim of this study was to investigate the value of platelet indexes including platelet count (PLT), MPV and PDW as diagnostic biomarkers for APE in Emergency Department (ED).

Material and methods

Study population

We retrospectively reviewed the medical records of all adult patients with suspected APE who were admitted to the Emergency Department of the Clinical Hospital No. 2 in Rzeszów in the period from July 2014 to February 2017. The initial evaluation of the patients included clinical history, symptoms, physical examination, hemogram parameters, 12-lead electrocardiography and D-dimer plasma testing. The Wells score was used for the prediction of APE.[14] In patients with suspected high-risk APE (in the presence of shock or persistent hypotension) CT angiography was immediately performed. The rest of patients were tested for D-dimer plasma level and in the case of increased level – CT angiography was accomplished (in accordance with 2014 ESC Guidelines on the diagnosis and management of APE).[14]

Smoking was defined as the inhalation of the smoke of burning tobacco in the amount of at least one cigarette per day. Diabetes mellitus was defined in accordance with the European Association for the Study of Diabetes criteria.[15] Arterial hypertension was defined as a systolic/diastolic blood pressure ≥ 140/90 mmHg or current use of antihypertensive treatment.[16] Family history of VTE was defined as a confirmed VTE episode in a first-degree relative. Obesity was defined as body mass index (BMI) ≥ 30 kg/m^2. Immobilization was defined as a bed rest ≥ 2 days. Hospitalization was defined as a hospital stay during the last 30 days.

Blood samples

Blood samples were collected from an antecubital vein on admission. EDTA- tubes were used for automatic blood count. The blood count was measured by a Siemens high volume hematology analyzer ADVIA 2120i (Siemens Healthcare Diagnostics, Eschborn, Germany). Sodium citrate tube and ACL TOP 500 analyzer (Beckman Coulter, Brea, CA, USA) were used for quantitative D-dimer measurement. Age adjusted cut-off values (age × 10 ng/ml) for patients over 50 years of age and 500 ng/ml for other patients were set. As a hospital policy, platelet indexes were measured within 1 hour after sampling.

Computed tomography

Multi-detector CT angiography was performed with the use of GE Revolution 256-slice scanner (General Electric Company, Boston, MA, USA) and GE Discover CT750 (General Electric Company, Boston, MA, USA).

Statistical analysis

Data were analyzed using SPSS software ver. 19.0 for Windows (SPSS Inc., Chicago, IL, USA). In order to identify the normal distribution, the Kolomogorov-Smirnov and Shapiro-Wilk tests were applied. Categorical variables were analyzed using the chi-square test or the Fisher's exact test (as appropriate). Student's t-test was used for variables with normal distribution and the values were presented as mean ± standard deviation (SD). Continuous variables without normal distribution were analyzed using the Mann-Whitney U test and the obtained values were presented as median (50th) values and interquartile ranges (25th and 75th). The Pearson correlation test (Pearson's r) were calculated for correlation of parametric variables and the Spearman's rank correlation test was used for nonparametric variables. The level of significance for the two-tailed p-value was set below 0.05 and confidence intervals (CI) were 95%.

Results

The baseline characteristics and laboratory measurements of patients are compared in Table 1. There were no intergroup differences in demographic or clinical variables. 145 patients were included in this study, with an average age 67 ± 18 years, 61% women. The average age of patients was similar in both of the groups (67 ± 17 vs. 67 ± 19; p=0.613). CT angiography was positive in 45% of cases (32.5% women vs. 54% men). APE was diagnosed with similar frequency in the female (54%) and the male sex (46%).

There were no significant differences in PLT (220 ×10^3/mm^3 [172-274] vs. 243 × 10^3/mm^3 [186-286],

Table 1. Characteristics of the studied groups

	Positive CT angiography (n=65)	Negative CT angiography (n=80)	p-values
Age, y	67 ± 17	67 ± 19	0.613
Male gender, n (%)	30 (46%)	26 (32.5%)	0.093
Body mass index, kg/m^2	26.98 ± 3.25	27.46 ± 3.75	0.415
Risk factors and comorbidities, n (%)			
Smoking	18 (28%)	17 (21%)	0.367
Oral contraceptives or HRT	0	0	1
Arterial hypertension	22 (34%)	31 (39%)	0.542
Family history of VTE	7 (11%)	4 (5%)	0.192
Diabetes mellitus	11 (17%)	13 (16%)	0.914
Obesity	15 (23%)	16 (20%)	0.653
Known malignancy	8 (12%)	4 (5%)	0.112
Immobilization	3 (5%)	1 (1%)	0.219
Hospitalization	6 (9%)	3 (4%)	0.174
Pregnancy or postpartum period	2 (3%)	0 (0%)	0.199
Laboratory parameters			
PLT [10^3/mm^3]	220 (172-274)	243 (186-286)	0.122
MPV [fL]	8.0 (7.6-8.4)	7.9 (7.4-8.7)	0.447
PDW [%] mean ± SD	59.0 ± 6.9	57.2 ±7.3	0.119
Hemoglobin [g/dL]	13.7 (12.1-14.4)	12.9 (11.7-14.0)	0.057
WBC [10^3/mm^3]	9.39 (7.8-13)	9.07 (7.19-12.73)	0.472
Creatinine [mg/dL]	0.90 (0.74-1.09)	0.89 (0.75-1.26)	0.905
NT-pro-BNP [pg/mL]	1434 (242-5460)	no data	-
Troponin T [pg/mL], cut-off value 14 pg/mL	27 (9-61)	no data	-
D-dimer [ng/mL]	7483 (3202-16878)	4874 (1935-16727)	0.17

Data are shown as median (interquartile range), mean ± standard deviation or number (percentage).
Abbreviations: HRT, hormone replacement therapy; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; MPV, mean platelet volume; PDW, platelet distribution width; PLT, platelet count; WBC, white blood cells;

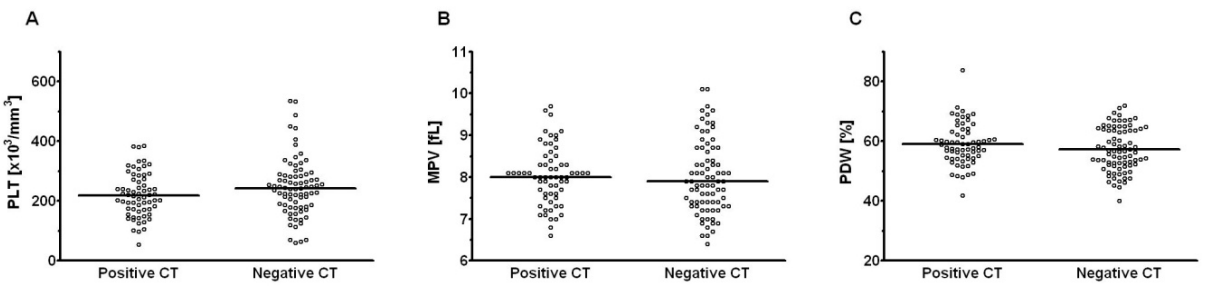

Fig. 1. Comparison of selected parameters in the studied groups. Horizontal lines represent medians (PLT and MPV) or means (only PDW) of each groups

p=0.122)(Fig.1A) and MPV (8.0 fL [7.6-8.4] vs. 7.9 fL [7.4-8.7], p=0.447)(Fig.1B).

The PDW did not differ between the patients with APE and the control group (59.0% ± 6.9 vs. 57.2 ± 7.3%, p=0.119)(Fig.1C).

No differences between the groups were found in other hematological parameters. Hemoglobin levels were similar (13.7 g/dL [12.1-14.4] vs. 12.9 g/dL [11.7-14.0], p=0.057). White blood cell (WBC) levels did not differ between the groups (9.39 × 10^3/mm^3 [7.8-13] vs. 9.07 × 10^3/mm^3 [7.19-12.73], p= 0.472)(Table 1).

Patients with positive CT angiography had an elevated level of N-terminal pro-brain natriuretic peptide (NT-pro-BNP) (1434 pg/mL [242-5460]) and T-troponin (27 pg/mL [9-61]; High-sensitive assay; cut-off value 14 pg/mL).

D-dimer level was increased in all individuals but there was no difference between the groups (7483ng/mL (3202-16878) vs. 4874ng/mL (1935-16727), p=0.17)(Table 1).

There was a positive correlation between MPV and PDW (r=0.638, p<0.001) likewise the creatinine level and age (r=0.345, p<0.05). Negative correlation was found between PLT and MPV (r= -0.334, p<0.001) and between PLT and PDW (r= -0.308, p<0.001).

In the group of patients with confirmed APE 7 (11%) patients met the criteria for high-risk PE and 4 patients died (6%). We found no differences with regard to the platelet indexes between high-risk PE patients and others, as well as between survivors and non-survivors.

During the collection of blood samples, none of the patients had international normalized ratio (INR) > 1.2 (data not shown) and none of them declared ongoing anticoagulant treatment.

Discussion

In this study we investigated the platelet indexes among patients with suspected APE in ED. However, we did not observe any differences between the APE group and others regarding to these parameters.

Recent publications showed the discrepancies in results concerning the role of MPV in APE and DVT at diagnosis. Varol et al. showed that MPV was increased among patients with APE whereas Lippi et al. supported an inverse association between MPV and the risk of VTE.[17,18] There were differences in the rules of inclusion criteria and control group between those studies. According to the *Quality Assessment of Diagnostic Accuracy Studies* (QUADAS) in our research the spectrum of patients included those, who will eventually receive a new diagnostic test in practice (patients with suspected APE in ED).[19] Moreover, we measured platelet indexes within 1 hour after sampling. It is important because delaying this test may influence the MPV when EDTA is used. There is a study which revealed that MPV can be accurately measured by both methods of anticoagulation (EDTA and sodium citrate) if analysis is performed within 1 hour after sampling.[20] Failure to comply with these rules may be the cause of existing differences.

Recently, platelets to lymphocyte ratio (PLR) was found to be a predictor of VTE and proved to be associated with the severity and long-term outcomes in patients with APE.[21-23] Further studies on larger populations are necessary.

In our study we found a negative correlation between PLT and MPV (r= -0.334, p<0.001). Platelets are involved in thrombus formation. Large platelets are immature, more adhesive and likely to aggregate than small ones.[24] Their number increase in case of platelets consumption and good bone marrow compensatory function. MPV is a parameter of volume and regeneration of platelets.

Among patients with positive CT we found elevated troponin and NT-pro-BNP. It can be associated with the right ventricular dysfunction among patients with an APE and it was found to be a significant predictor of mortality in the above-mentioned individuals.[25]

Positive predictive value of elevated D-dimer is low and it is not useful for confirmation of APE. In this study we confirmed the low specificity of D-dimer in APE diagnosing. There was no significant difference between the group with positive and negative CT angiography (p=0.17). Pulmonary embolism is pathophysiological pulmonary circulation disorder syndrome caused by partial or complete occlusion of the pulmonary artery and it is commonly a consequence of deep vein thrombosis. The main cause of death in severe stage is acute right ventricle failure due to pressure overload. Thrombosis begins with the aggregation of erythrocytes, fibrin and platelets. D-dimer is a sensitive marker of acute thrombosis because of simultaneous activation of coagulation and fibrynolysis.[14] The specificity of D-dimer is poor because fibrin is produced in a variety of conditions such as cancer, trauma, inflammation, pregnancy and infection. [14]

Our study has several limitations. Not all of the comorbidities and environmental factors that might have affected platelet count and functions were taken into account. MPV is believed to be a marker of platelet activity but it is not specific. It was a single-center, retrospective study and included a relatively small sample size.

Conclusion

To conclude our results, it can be noted that platelet indexes (at a single time point) are not a reliable indicator for the diagnosis of APE in the Emergency Department. Further studies in this matter are necessary.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.2

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 118–122

ORIGINAL PAPER

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Balance evaluation after Russian current on the femoral rectus of healthy individuals

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ABSTRACT

Introduction. A technique used in physiotherapy, but still underinvestigated, is the use of the Russian current as an aid in the improvement of balance.

Aim. To verify the influence of the Russian current applied to the rectus femoris on balance in healthy and sedentary individuals.

Material and methods. A cross-sectional clinical trial was performed at the Universidade Estadual do Oeste do Paraná – Unioeste, in the city of Cascavel – PR. The sample consisted of 20 healthy female subjects aged between 18 and 25 years, equally divided into two groups where group 1 was placebo and group 2 treatment. Initially, the proprioceptive evaluation was performed by means of a functional test (the Star Excursion Balance Test (SEBT)) and stabilometry using a baropodometer. Russian current was then applied to the femoral rectum of both limbs simultaneously for 2 weeks, 5 days a week.

Results. No significant differences were found analyzing the variables, but the elevated effect size points to clinical relevance of Russian Current in functional assessment.

Conclusion. The use of the Russian current in the rectus femoris did not present significant alteration on balance.

Keywords. knee, physical therapy, proprioception, range of motion

Introduction

A property that, when diminished, is related to an increased risk of injury is proprioception, defined as a somatic sensation that encompasses the knowledge of joint movement (kinesthesia) and also of joint position (joint position sense), corpuscles of Paccini and Meissner, muscle spindles, Ruffini terminations, and the Golgi tendon organ are musculoskeletal afferent structures

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 30.04.2019 | Accepted: 08.05.2019
Publication date: June 2019

Czarnobaj CC, Folarosso HC, Picagevicz MG, de Lima N, Kiyosen Nakayama G, Flor Bertolini GR. *Balance evaluation after Russian current on the femoral rectus of healthy individuals.* Eur J Clin Exp Med. 2019;17(2):118–122. doi: 10.15584/ejcem.2019.2.2

responsible for sensation, and physical exercise through muscle contraction can alter their responses.[1–3]

Within the physiotherapeutic field, a form of electrical stimulation gained popularity from reports by the Russian physiologist Yakov Kots, who argued that the medium frequency current at 2500 Hz, modulated at low frequency, increased the recruitment of motor units during muscle contraction, gaining over 40% of what would happen in a voluntary contraction. Because of its relatively high frequency, one of the main advantages is its better tolerability, however, the literature is not clear if it actually produces greater gains than low frequency stimulation.[4,5]

When the muscle contraction is used therapeutically, it is sometimes interesting the phenomenon of reciprocal inhibition, which occurs when the agonist muscle group of a certain movement is activated, the antagonist group undergoes a relaxation, this may aid in the gain of muscular extensibility, altering the agonist-antagonist contraction ratio.[6-8] Furthermore, the use of electrostimulation has shown to be promising in peripheral nerve lesions and also as a factor to improve proprioception in central nervous lesions.[9,10]

Considering that the Russian current is not yet a fully exploited form of electrostimulation, especially with respect to alterations in balance, the objective of this article was to verify if its use on the rectus femoris muscle could generate changes in the balance of healthy and sedentary youngsters.

Material and methods

This study is characterized as a random clinical trial, transverse, with a quantitative character. The study was carried out at the Centro de Reabilitação Física (CRF) of the Universidade Estadual do Oeste do Paraná – Unioeste. The sample, selected for convenience, by direct invitation, consisted of 20 healthy young women, 18 to 25 years were recruited to evaluate. These were divided equally, by means of an opaque envelope, into two groups: a placebo group (PG) and treatment group (TG).

The inclusion criteria were: not to practice physical activity regularly; have no contraindication to electrostimulation and agree to voluntarily take part in the research. The exclusion criteria were as follows: alcoholism and/or smoking; having fractured lower limbs; low back pain; practice stretching; neurological deficits; have any contraindication to the use of electric currents and lack any collection. The application of the Russian Current occurred for two weeks, 5 days each week. The participants were assessed on the first day before the application of the current (EV1), after one week of intervention (EV2) and at the end of the second week (EV3). After two weeks of follow-up, the participants were re-evaluated (EV4). They were also made aware of the research procedures and signed a Free and Informed Consent Term, previously approved by the Research Ethics Committee of Unioeste under number 2,162,807.

The proprioceptive evaluation was performed through a functional test, the Star Excursion Balance Test (SEBT) and stabilometry using a baropodometer. The SEBT is a test that evaluates the dynamic postural equilibrium, requiring that the unipodal balance be maintained while performing pre-determined range movements with the contralateral limb.[11] As a guide, adhesive tapes were glued to the ground in 8 directions, each one 120 cm long. The lines were arranged in a star at 45º and were named according to their direction from the inferior support member (intersection of lines): anterolateral (AL); anterior (ANT); anteromedial (AM); medial (MD); postero-medial (PM); posterior (PO); posterolateral (PL) and lateral (LAT).

To perform the test initially the volunteers remained at the point of intersection of the eight lines in bipodal support. They were then instructed to touch lightly with the toe of the contralateral limb (free limb) as far as possible on each of the eight lines (directions), and return to bipodal support, this distance being recorded. It was discarded if the volunteer removed the lower support limb from the center of the figure, or was unable to keep balance during the test. Before the individual performed the test, the examiner performed the explanation and demonstration of the procedure, and it was done bilaterally in three attempts, noting the highest value reached. All participants began with their left foot in the central position of the intersection of the lines.

Static balance was evaluated by the baropodometer through stabilometry that documents the analyzes with images of plantar pressure points in a modular platform constituted by electronic sensors that recognize the information of the support, conserving the natural mobility, and analyzed through the Footwork program®. Quantifying the anteroposterior and lateral oscillations of the body, per cm^2 and load in %. It was performed with the subject in the orthostatic posture on the platform, in bipodal support, the upper limbs in the prolongation of the body.[12,13]

In the interventions, they were submitted to the Russian current for 10 minutes, using the following parameters: a carrier frequency of 2500 Hz, modulated frequency of 50 Hz. Sine wave with synchronized stimulation, rise and fall time equal to 1 second, contraction time (On) of 6 seconds with a timeout of 7 seconds. The intensity of the stimulus was adapted according to the maximum tolerable level, always with visible muscular contraction. The current was applied bilaterally with the participant in dorsal decubitus (DD), with knee extension and without associated voluntary contraction. An electrode was placed in the femoral rectus muscle at 5 cm above the upper edge of the patella and the other electrode was placed on the motor point of the

same muscle of each patient (individually tested prior to the start of electrostimulation, as the point obtained the more vigorous contraction with the same intensity). Positioning was similar for the placebo group, but no flow was achieved.

The data analysis was quantitative and the data were analyzed through descriptive and inferential statistics. Unidirectional ANOVA was utilized and the normality of the data was observed by the Shapiro-Wilk test. Quantitative variables were characterized by mean and standard deviation. In all cases, the accepted level was 5% ($p < 0.05$). The effect size (ES) analysis of Cohen was also carried out in accordance with the following classification: <0.2 trivial; 0.2-0.5 small; 0.5-0.8 moderate; >0.8 large. ES assessments were always based on EV1 within their own group.

Results

Twenty volunteers met the study inclusion criteria, two of them being excluded because they did not attend the data collection, and 9 volunteers remained in each group. The mean age of participants was 21.33 ± 1.7 years.

For the SEBT test, there were no significant intragroup differences in row or mean direction as well as in the same direction between groups ($p > 0.05$). However, when checking Cohen's analysis, it was possible to observe that most of the effect sizes were trivial or small for PC, whereas in the treated group there were a predominance of moderate and large effect sizes (table 1).

Data for the analysis of mean pressure (kPa), maximum pressure (kPa), surface (cm), previous distribution (%), posterior distribution (%) and pressure center position (COP in centimeters) acquired by the baropodometry data analysis in each evaluation, also did not present differences ($p > 0.05$) intra or between groups; and overall effect sizes were trivial and small for both groups (table 2).

Discussion

In the present study, we attempted to test the isolated action of the Russian current on part of the quadriceps (femoral rectus), in a possible production of proprioception changes, both by functional evaluation and by an instrumentalized evaluation method (baropodometry). It was not possible to observe any significant change over time, or in comparison with a control group, but with larger effect sizes for the evaluation of the SEBT for the treated group, indicating clinical effects for the current. The Russian current despite reports of higher gains in muscle strength, has not shown to be advantageous over other forms of electrostimulation for the production of torque, force gain or even pleasantness.[5,14-21]

Evaluation of proprioception, which is part of the body balance, is a complex and difficult activity, since many factors can influence changes in postural stability under normal and pathological conditions.[22] One of the ways to evaluate is the SEBT instrument, because it is a balance test considered as current tool, easy to handle, non-instrumental and cost-effective.[11] Peres et al. evaluated 11 healthy volleyball athletes through the SEBT, after a four-week proprioceptive training program, observing improvement in six directions on the right ankle and five on the left ankle.[23] Braga et al. proposed a proprioceptive training, with Nintendo Wii or proprioceptive disc, for young and healthy women, evaluated by the SEBT, both of which showed an improvement in the performance of the SEBT.[24]

In relation to the use of the baropodometer in sedentary young adults, it is an instrumentalized way of evaluating pressure distribution of the foot and pressure center, in which several variables can be measured, such as static balance and proprioception.[13] Da Silva et al. used this instrument to evaluate the effect of the low-power infrared laser, applied to the muscles of the posterior leg compartment, not observing proprioceptive changes for the sample.[12] Alfieri, Teodori and Guirro observed that a program of regular physiotherapeutic intervention in the elderly was able to increase the area of plantar distribution and reduction of peak pressure in bipodal support.[25]

According to Hara the improvement of motor function in patients after stroke, is most effective when the electrostimulation is initiated by electromyographic signal than when used spontaneously.[26] Since functional electrical stimulation (FES) induces greater muscle contraction when compared to voluntary contraction. Still, proprioceptive feedback may play a significant role in this FES assisted therapy. Bustamante et al. stimulated FES (50 Hz, 300 μs) flexor and wrist extensor muscles, a patient with 11-month sequelae of hemorrhagic stroke for 1 hour daily for 10 days, associating FES assisted workout movements.[10] They evaluated proprioception through the joint position sense test, and report that there was improvement in both angles and time to carry out the task for the electrostimulated wrist. It should be emphasized that when comparing with the present study, there was no activity other than electrostimulation for the quadriceps, and yet, the volunteers were all healthy, and a possible positive effect of electrostimulation on proprioception may depend on a deficit since according to Christensen and Grey the electrical stimulation is used as a therapeutic modality in motor rehabilitation to effect movements that could be difficult to perform by voluntary activation only.[27,28]

Thus, it is observed as a limitation that the population of the present study is composed only by healthy youngsters, which also limits the action of the electrostimulation; another limitation of the present study was the small sample size used, which may have interfered with the presented results of the statistical analysis; and it is therefore suggested that new studies should address with larger sample sizes and populations with some type

Table 1. Mean values and standard deviation for the SEBT evaluation of the Placebo group (PG) and Treatment (TG), distance measured in centimeters, according to the different moments of evaluation (EV), below the mean values the effect size values are presented, based on the EV1 of the same group

	PG				TG			
	EV1	EV2	EV3	EV4	EV1	EV2	EV3	EV4
ANT	57.6±5.1	57.2±5.4 0.33	57.5±7.1 0.08	56.0±4.5 -0.02	56.4±6.3	58.8±5.4 0.41	59.6±3.8 0.62	60.9±4.1 0.85
AL	58.5±5.4	58.5±5.3 0	59.4±6.7 0.15	56.4±4.3 -0.43	57.8±4.7	59.7±4.9 0.40	61.4±3.7 0.85	61.1±4.2 0.74
LAT	58.9±4.7	59.0±4.7 0.02	58.4±6.6 -0.09	56.5±3.8 -0.56	57.8±5.5	58.9±5.4 0.20	61.5±4.6 0.73	60.5±4.9 0.52
PL	57.0±6.0	57.0±5.9 0	59.9±6.0 0.48	57.8±3.9 0.16	58.8±4.8	59.3±5.6 0.10	61.4±5.4 0.51	59.9±4.8 0.23
POS	55.0±5.7	55.0±6.0 0	58.3±5.7 0.58	54.5±5.3 -0.09	52.9±4.4	57.0±5.9 0.79	58.0±5.5 1.02	57.4±4.8 0.98
PM	53.0±9.3	53.2±9.3 0.02	57.8±6.5 0.60	53.9±7.1 0.11	48.3±6.4	54.8±5.9 1.06	55.9±5.7 1.25	56.0±4.2 1.42
MED	47.9±6.6	47.9±7.0 0	51.9±5.6 0.65	49.0±4.4 0.20	47.6±6.8	50.9±4.7 0.56	52.2±7.1 0.66	51±4.9 0.57
AM	55.3±5.6	55.3±6.0 0	56.6±6.6 0.21	54.2±4.7 -0.21	54.7±7.0	56.2±5.2 0.24	57.0±5.5 0.37	57.3±4.9 0.43
Mean	55.4±4.9	56.0±4.1 0.13	57.5±5.4 0.41	54.8±3.7 -0.14	54.3±4.5	55.9±4.5 0.36	58.4±4.3 0.93	58.0±3.6 0.91

ANT – anterior; AL – anterolateral; LAT – lateral; PL – posterolateral; PO – posterior; PM – posteromedial; MD – medial; AM – anteromedial

Table 2. Mean and standard deviation values for the baroscopic evaluation of the Placebo (PG) and Treatment (TG) groups, according to the different moments of assessment (EV), below the mean values the effect size values are presented, based on the EV1 of the same group

	PG				TG			
	EV1	**EV2**	**EV3**	**EV4**	**EV1**	**EV2**	**EV3**	**EV4**
AP	31.4±7.2	31.0±4.7 -0.07	30.3±7.1 -0.15	31.4±5.4 0	32.7±6.3	32.8±7.0 0.02	31.8±6.8 -0.14	33.6±6.6 0.14
PMáx	105.9±40.3	107.8±25.6 0.06	118.1±37.8 0.31	117.1±30.6 0.31	107.8±24.1	115.6±28.5 0.26	104.4±21.3 -0.13	113.6±22.4 0.22
Sup	67.2±13.4	72.5±13.7 0.39	72.0±15.5 0.33	78.6±14.7 0.81	59.0±9.0	62.5±8.6 0.40	62.1±11.5 0.41	70.9±11.7 1.14
AD	20.5±9.3	22.5±6.2 0.25	21.2±8.6 0.08	20.8±4.8 0.04	20.9±6.6	19.4±8.2 -0.20	20.9±6.4 0	21.2±4.4 0.05
PD	29.5±7.9	27.5±7.5 -0.26	28.5±10.1 -0.11	29.2±7.2 -0.04	29.1±7.6	30.6±9.4 0.18	29.0±8.3 -0.01	28.8±5.2 -0.05
COP	8.5±2.4	8.9±1.5 0.20	8.9±1.9 0.18	8.7±1.8 0.09	8.6±1.2	8.9±1.9 0.19	8.5±1.7 -0.07	8.4±1.1 -0.17

AP – average pressure (kPa); MaxP – Maximum pressure (kPa); Sup – Superficies (cm²); AD – anterior distribution (%); PD – posterior distribution (%); COP – center of pression (cm)

of motor deficiency and the repercussion of the Russian current on samples of these. The results showed that the use of the Russian current in the rectus femoris muscle did not show significant changes in knee proprioception, but clinically presented functional results superior to placebo.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.3

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 123–130

ORIGINAL PAPER

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Determinants of women's behavior in breast cancer prevention

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ABSTRACT

Introduction. According to statistics, breast cancer is the second leading cause of death in Poland. Progress in treatment and diagnosis gives an opportunity of a quick diagnosis, but women are reluctant to undergo prophylaxis screening.

Aim. To identify women's attitudes about breast cancer prophylaxis.

Material and methods. The diagnostic survey was conducted in a group of 200 women. The research tool was the questionnaire developed by the authors.

Results. Knowledge about breast cancer and prophylaxis was on an average level. Nearly half of the women surveyed (45.5%) declared that they do not ask for a breast examination while visiting a gynecologist. Only 26.8% of the surveyed women were systematically subjected to preventive examinations for breast cancer, while 18.7% of them performed breast self-examination. Every fifth respondent used invitation for a free mammogram.

Conclusion. The women's knowledge about breast cancer is average, but it does not translate into their attitudes towards the prevention of this cancer. Most women niether perform breast self-examination nor benefit from free prophylaxis programs. Younger respondents most often use the Internet. Education and place of residence do not affect the participation of women in preventive examinations for breast cancer.

Keywords. attitudes, breast cancer, knowledge, prophylaxis

Introduction

Nowadays, breast cancer is a major health issue. Similar to lung cancer, it accounted for 12.3% of newly diagnosed cancers in the world in 2018.[1] What is also disturbing is the steady increase in the incidence in Poland. The incidence rate in 1999 was 38.78/100,000, while in 2009 this level increased to 50.37/100 thousand, and in 2018 – 59.1/100,000. Mortality in this period was at the level of 14/100,000. According to the National Cancer Registry in 2016, breast cancer was the second leading cause of death among women in Poland after lung cancer.[2]

The incidence exceeds the number of deaths three times, which proves the progress that has taken place over the years. The 5-year survival rate also reached

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 23.03.2019 | Accepted: 29.04.2019
Publication date: June 2019

Nagórska M, Aksamit M, Krygowska K, Bassara-Nowak D. *Determinants of women's behavior in breast cancer prevention*. Eur J Clin Exp Med. 2019;17(2):123–130. doi: 10.15584/ejcem.2019.2.3

a high level of 75% thanks to the introduction of the breast cancer screening program, effective diagnosis and treatment.[3-5]

The prevalence of breast cancer is not the same in all countries, the difference between the lowest and the highest prevalence varies 25 times. About 55% of breast cancer cases are reported in industrialized countries, with a similar percentage of deaths reported in developing countries.

The highest prevalence of breast cancer (90–100/100,000) is recorded in North America, Australia, northern and western Europe. The average prevalence is observed in Central and Eastern Europe. The lowest incidence rate (27/100 000) is recorded in Africa and Southeast Asia. In 2012, 1.67 million of new breast cancer cases were diagnosed, which is 25.2% of all cancers in women, and more than 2 million new cases were diagnosed in 2018. 626,679 deaths due to breast cancer were confirmed.

Mortality, like morbidity, varies. Southern part of South America, Africa and Asia are the areas where the highest mortality due to breast cancer was recorded, while Central America and some regions of Asia have the lowest mortality rate.[6]

The risk factors increasing the incidence are: 50–70 years of age, early menarche age, menopause at late age, birth of the first child after 30 years, long-term hormone replacement therapy, family history of cancer and mutations in the BRCA1, BRRCA2 genes.

Primary prevention in breast cancer is based on the implementation of a healthy lifestyle, first of all – a healthy diet and physical activity, and maintaining a normal body weight.[7,8] Breastfeeding is an important factor in reducing the risk of getting sick. It has been proven that long-term> 6-month breastfeeding may reduce the risk of disease by 10–30%.[9] Other preventive measures, resulting from the fact that 3–8% of breast cancers are genetically determined, are chemoprevention and prophylactic mastectomy and / or ovariectomy. These are treatments dedicated to people with mutations in the BRCA1 and / or BRCA2 genes, in whom the risk of breast cancer amounts to 55–85% and 37–85%, respectively. The risk of developing ovarian cancer in these people is 15–60% and 15–27%, respectively.[10-13]

Secondary prevention, i.e. all actions aimed at early detection of an already existing disease, includes: mammography in healthy women and ultrasonography as a complementary examination of breast self-examination.

The screening system for breast cancer in Poland includes a population of women aged 50–69 years, in whom mammography can be performed every 2 years. However, in women from the risk group (breast cancer among the closest family members, mutation within BRCA1 or BRCA2 genes), a mammography re-screening should be performed after 12 months.[14]

Aim

The aim of the paper was to identify women's attitudes about breast cancer prophylaxis.

Material and methods

The study used the method of a diagnostic survey, a survey technique. The research tools were a questionnaire developed by the authors, which contained twenty-seven questions. Fifteen of them checked the women's knowledge about breast cancer prevention. One point was awarded for each correctly answered answer. In order to assess the level of knowledge, the following scores were adopted: 7 points – low level of knowledge, 8–12 points – moderate, 13–15 points – high. The remaining questions concerned attitudes towards breast cancer prevention. Statistical analysis was carried out using the SPSS software. The chi-square independence test was used for statistical analysis, and if the relationship between the variables was confirmed, the V Kramer's test determining the strength of dependence was additionally used.

Characteristics of the study group

The study was carried out at the turn of February and March 2015. The group of respondents consisted of 200 adult women, patients of the Medical Care Center in Jarosław, who voluntarily gave consent to participate in the study. Survey questionnaires were handed personally after providing information about the purpose of the study, the way of completing it and anonymity. Correctly completed questionnaires were tantamount to the consent to participate in the study.

The most numerous group were women aged 41–50, slightly smaller, but equally numerous group were women over 50. The remaining 17.5% were women aged 31–40, the least numerous group were women aged 19–30 (14%). The research shows that 53.3% of women were rural residents, while 46.7% of women lived in the city.

Among the respondents, 72.5% were married women, 17.5% were single, and only 10% were widows. The respondents most often had higher education (45.5%), every fifth (23.2%) – vocational education, 17.7% – secondary, and 13.6% – primary education.

The women performing mental or manual work accounted for 45.5%, the remaining 9% were retired or on pension.

A large proportion of women surveyed (40%) gave birth to 1 or 2 children, a little less (32.5%) – 3 children. Nulliparous women constituted 18.5% and only 9% gave birth to more than four children.

Among the women surveyed, the vast majority (93%) had their first menstrual period between the age of 12 and 15. In the study group, 36% gave birth to their first child at the age of 21–25, slightly less (34%) – at the age of 17–20, in the third place were women giving birth

Table 1. The characteristics of the studied population

	Variable	N	%
Age	19-30	28	14.0
	31-40	35	17.5
	41-50	72	36.0
	> 50	65	32.5
Marital status	Married	145	72.5
	Widow	20	10.0
	Single	35	17.5
Number of children	None	37	18.5
	1-2	80	40.0
	3-4	65	32.5
	> 4	18	9.0
Age at the birth of the first child	17-20	68	34.0
	21-25	72	36.0
	26-30	38	19.0
	>30	22	11.0
Age at menarche	12-15	185	93.0
	16-18	14	7.0
Education	Higher	90	45.5
	Secondary	35	17.7
	Vocational	46	23.2
	Primary	27	13.6
Type of work performed	Mental	90	45.5
	Physical	90	45.5
	Retired	18	9.0
Source of knowledge	Internet	101	50.5
	Books	100	50.0
	Medical staff	45	22.5
	Other sources	24	12.0
Place of residence	Urban area	93	46.7
	Rural area	106	53.3

to the first child at the age of 26–30 (19%), the least numerous was a group of women that had their first child above 30 years of age (11%).

Over half of the respondents indicated the media – the Internet, radio or TV as a source of information about breast cancer. Few less got knowledge from scientific books and press, and one in five was educated by medical staff (Table 1).

Results

The results of the test of knowledge indicate that the largest group (81.5%) were the subjects who presented average level of knowledge (8–12 points), 14.5% women presented a low level of knowledge (7 points). The highest score (13–15 points) obtained only 4% of the respondents.

Among the surveyed women, 62.1% of the respondents confirmed that they regularly undergo prophylaxis screening for breast cancer, 37.9% did not regularly screen.

Most women did not perform self-examination of the breast (58.6%), while 31.3% of the respondents declared performing self-examination of the breasts.

The largest group of respondents (91%) agreed that women with the family history of breast cancer should be covered by prophylaxis – the answers "yes" and "rather yes", but 8.0% claimed that such women should not be subjected to prophylaxis.

The research showed that more than half of the respondents (65.5%) had the mammograms performed, 34.1% of the patients had never the mammography performed. A large proportion of the respondents (89.7%) reported to a gynecologist, when they detect a nodule during breast self-examination, 10.3% of women ignored this fact.

Nearly two-thirds of women (65.5%) felt that breast cancer screening programs should be more publicized in the media.

When asked whether women with breast cancer should be offered support group meetings, the vast majority of women (75.5%) answered "yes" and "rather yes".

Table 2. Level of knowledge of women and attitudes towards breast cancer prevention

What sources of knowledge do you use about breast cancer?			Women's knowledge of breast cancer (max 15 points)			Total
			7 pts.	8-12 pts.	13 - 15 pts.	
1			*2*	*3*	*4*	*5*
Internet, radio, TV	Yes	N	19	77	5	101
		%	65.5	47.2	62.5	50.5
p = 0.15						
Scientific books, press	Yes	N	7	88	5	100
		%	24.1	54.0	62.5	50.0
P = 0.01, Kramer's V = 0.26, Chi – square = 9.30 (df = 2)						
Medical professional	Yes	N	8	36	1	45
		%	27.6	22.1	12.5	22.5
P = 0.64						
Other source	Yes	N	1	23	0	24
		%	3.4	14.2	0	12.0
P = 0.15						
Do you regularly undergo preventive examinations for breast cancer?	Yes	N	8	44	1	53
		%	27.6	27.3	12.5	26.8
	Rather yes	N	10	52	3	65
		%	34.5	32.3	37.5	32.8
	No	N	6	26	2	34
		%	20.7	16.1	25.0	17.2
	Rather no	N	5	39	2	46
		%	17.2	24.2	25.0	23.2
P = 0.93						
Do you regularly perform breast self-examination?	Yes	N	3	32	2	37
		%	10.3	19.9	25.0	18.7
	Rather yes	N	9	40	0	49
		%	31	24.8	0	24.7
	No	N	11	57	4	72
		%	38	35.4	50.0	36.4
	Rather no	N	6	32	2	40
		%	20.7	19.9	25.0	20.2
P = 0.62						
Have you ever had a mammogram??	Yes	N	19	81	5	105
		%	65.5	50.3	62.5	53.0
	No	N	8	80	3	91
		%	27.6	49.7	37.5	46.0
	Rather no	N	2	0	0	2
		%	6.9	0	0	1.0
P = 0.06						
If you detect a lump during self-examination, do you report to the doctor?	Yes	N	12	91	4	107
		%	41.4	56.5	50.0	54.0
	Rather yes	N	14	51	4	69
		%	48.3	31.7	50.0	34.8
	No	N	1	7	0	8
		%	3.4	4.3	0	4.0
	Rather no	N	2	12	0	14
		%	6.9	7.5	0	7.1
P = 0.60						

1			*2*	*3*	*4*	*5*
Do you lead a healthy lifestyle?	Yes	N	4	20	0	24
		%	13.8	12.4	0	12.1
	Rather yes	N	5	84	6	95
		%	17.2	52.2	75.0	48.0
	No	N	15	24	2	41
		%	51.8	14.9	25.0	20.7
	Rather no	N	5	33	0	38
		%	17.2	20.5	0	19.2
P < 0.001, Kramer's V = 0.26, Chi – square = 26.46 (df = 6)						
If you are at the gynecologist and he does not perform a breast examination, do you ask for this examination?	Yes	N	7	30	0	37
		%	24.1	18.6	0	18.7
	Rather yes	N	3	33	2	38
		%	10.3	20.5	25.0	19.2
	No	N	18	68	4	90
		%	62.2	42.3	50.0	45.5
	Rather no	N	1	30	2	33
		%	3.4	18.6	25.0	16.7
P = 0.16						
When you feel the discomfort of the reproductive system, do you report to the gynecologist?	Yes	N	14	84	4	102
		%	48.3	52.2	50.0	51.5
	Rather yes	N	10	64	2	76
		%	34.5	39.8	25.0	38.3
	No	N	2	7	1	10
		%	6.9	4.3	12.5	5.1
	Rather no	N	3	6	1	10
		%	10.3	3.7	12.5	5.1
p = 0.55						

The research showed that 31% of surveyed women thought that they lead a healthy lifestyle, the remaining 69% admitted that they do not lead a healthy lifestyle.

The survey also asked about participation in prophylaxis screening, 38% of women admitted that they do not use invitations for free mammography, 22% of women answered that they are likely to take advantage of it, the answer "yes" was indicated by 21.5%, almost as many as the answer "rather yes". 17.5% of women indicated the answer "rather not".

The research shows that 65.6% of women surveyed did not ask for a breast examination during an appointment at a gynecologist, 34.4% asked for such an examination. In the group of women surveyed, 81.8% reported to a gynecologist when they experience some discomfort from the reproductive system.

Women with higher scores in the knowledge test preferred scientific books as their source of knowledge ($p = 0.01$). In the case of other sources of knowledge, no statistically significant relationships were found with the level of respondents' knowledge.

There was no statistically significant relationship between women's knowledge about breast cancer and regular prophylaxis screening for breast cancer ($p = 0.93$) as well as between women's knowledge and breast self-examination ($p = 0.62$).

There was a weak statistically significant relationship ($p = 0.06$) between women's knowledge about breast cancer prevention and mammography.

A lack of statistically significant relationship ($p = 0.60$) was found between knowledge and going to the gynecologist after detection of a lump in the breast during breast self-examination. The research showed that women who do not lead a healthy lifestyle are more often characterized by a lower level of knowledge about breast cancer. The dependence was weak ($p < 1$). There was no statistically significant relationship ($p = 0.30$) between knowledge and the use of invitations to free mammography by women. A lack of statistically significant relationship ($p = 0.16$) was found between knowledge and asking for breast examination by women, when a doctor does not perform it.

The results showed that there was no relationship ($p = 0.55$) between women's knowledge about breast cancer prevention and women reporting to a gynecologist when they experience some discomfort from the reproductive system. The relationship between the lev-

el of respondents' knowledge and their attitude towards breast cancer prevention is illustrated in Table 2.

Statistical analysis showed that younger women acquired knowledge more often from the Internet, radio and TV, this is a weak and statistically significant relationship ($p = 0.02$). On the other hand, older women acquired knowledge from other unmentioned sources – a weak statistically significant relationship ($p = 0.008$). There was no statistically significant relationship between the place of residence and the sources of knowledge about breast cancer. Internet, radio, TV ($p = 0.84$), scientific books, press ($p = 0.18$), medical professional (doctor, nurse, midwife) ($p = 0.74$) and other source ($p = 0.73$)). There is also a lack of dependence between the place of residence ($p = 0.11$) and education of the surveyed women ($p = 0.59$), and participation in preventive examinations for breast cancer.

Discussion

Breast cancer is one of the leading causes of death among women in Poland and in the world. In the initial stage of the disease, the possibility of curing is complete, in the late stage the chances become smaller.[15] In this situation, the women's knowledge is important, which often determines their attitudes towards the prevention of breast cancer. In the study, women most often presented average (81.5%) or low (14.5%) level of knowledge. The level of women's knowledge about breast cancer was also unsatisfactory in studies carried out by other authors in several countries around the world.[16-20]

In Ślusarska's study, 82.6% of women know the principles of breast self-examination, while in Najdyhor research - 57% of women, and in Węgrowski's study - 42% of the respondents could not indicate the optimal time for breast self-examination.[21-23] The correct answer to the question about optimal breast self-examination time (2-3 days after menstruation) in the present study gave 86% of the respondents, the remaining 14% considered the statement as false. In Abolfotouh's study over 40% of the respondents correctly indicated the frequency and optimal time of self-examination.[18]

The knowledge of the risk factors is necessary to prevent cancer. The Przysada and Najdyhor studies showed that the vast majority of women surveyed lacked knowledge about them.[16,22] In our study women mostly responded correctly.

An important aspect in the prevention of breast cancer is the knowledge of symptoms that indicate cancer. Women asked about the symptoms of breast cancer did not show much knowledge e.g. the question whether enlarged axillary lymph nodes are a symptom of breast cancer, 54.8% of them responded correctly. Almost half of the respondents incorrectly indicated that this is a false answer. Much better with a similar question coped the women in Ślusarska's study, where over 70% considered enlarged axillary lymph nodes as a sign of cancer. The question about the way of breast cancer spread was the most problematic. The vast majority of women (74.5%) indicated the wrong answer. Only 25.5% knew the correct answer. Similarly, in the studies of Woźniak, where 39% of respondents knew the symptoms of breast cancer, the remaining 56% did not have such knowledge.[24]

The second part of the study concerned the attitudes of women towards the prevention of breast cancer. Our study was in line with the results of other authors investigating similar aspects of women's attitudes towards breast cancer prevention.

In Ślusarska's studies, over 61% of the respondents knew that they should have a breast examination during the visit to a gynecologist, but they do not ask for it.[21] The results of our study are comparable, because 45.5% of women declared they would not ask for it if a gynecologist did not suggest this examination.

On the other hand, only 26.8% of the respondents who answered "yes" to the question about having systematic prophylactic examinations for breast cancer, 32.8% answered "rather yes" – these are quite low results. Breast self-examination, which is a fast, and also the cheapest method to detect early changes in mammary glands, is performed by only 18.7% of women. As many as 36.4% of women do not perform such test. For comparison, in Alwan et al. studies 42.6% of women perform breast self-examination (BSE).[17] Regular self-examination is performed by every third Greek woman.[26]

In Przysada study, the majority of women asked about breast self-examination confirmed doing it (81%), according to research by Woźniak, breast self-examination performs 54%.[16,24] After detecting the lesion, 89% of the respondents indicated that they reported to the doctor, for comparison in research among women in Ireland almost 70% saw a GP after detecting the lesion.[25]

The literature states that mammography is the most effective prophylactic examination for breast cancer. In our research, every second woman (53%) had mammography performed, which coincides with Przysada study, where 53% of women have undergone a mammographic examination at least once.[16]

The results of research carried out by the teams of Korkut, Dewal, Lostao and Garwacka-Czachor in Turkey, USA, Spain and Poland confirm that despite satisfactory knowledge of women about breast cancer, they do not participate in preventive examinations.[27-30] In our research were in line with them, i.e. the level of knowledge does not affect health-related behaviors - participation in screening tests.

In the Przysada study, a large proportion of the respondents were in favor of the need to implement and publicize preventive programs, which coincides with

the present research. 66.2% of women are in favour of adevertising breast cancer prevention programs in the media, 30.3% of the respondents answered "rather yes", only 1% of women indicated the answer "no".

However, statistics on the use of invitations for free mammography are still disturbing. Research shows that only 21.7% use such invitations, 22.2% rather use them, and 38.4% do not use them. This statistic looks similar to Przysada study, where 33% of the respondents took advantage of this possibility, and almost half did not, explaining it with the lack of necessity.[16]

According to the study, women do not have enough knowledge about breast cancer and the right attitudes towards prevention. They are still reluctant to use invitations for free mammography, disregarding their health. Knowledge and awareness as well as social support are essential elements in the prevention and treatment of breast cancer.

Conclusions

Summarizing, the women's state of knowledge about breast cancer is on average level, however, women's knowledge does not translate into their attitudes towards the prevention of this cancer. Most women do not perform breast self-examination and do not benefit from free prophylaxis programs. Moreover, the age of women determines the choice of sources of information about breast cancer. Younger respondents most often use the Internet.

Education and place of residence do not significantly affect the participation of women in prophylaxis screening for breast cancer.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.4

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 131–135

ORIGINAL PAPER

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Relative influence of body mass index and socioeconomic class on blood pressure levels and health

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ABSTRACT

Introduction. Blood pressure (BP) is a complex entity which is influenced by many factors. The impact of socioeconomic class and body mass index (BMI) on hypertension has been reported in the past but literature on their influence on blood pressure in healthy adolescents is very limited.

Aim. The aim of the study is to assess the influence of BMI and socioeconomic status on BP in healthy adolescents.

Material and methods. This cross sectional study includes three hundred healthy adolescents.

Anthropometric and BP measurements were done. BMI categories were derived using WHO Asia Pacific guidelines. An updated Kuppuswami scale was used for determining socioeconomic status.

Multiple regression analysis and analysis of variance was used to study impact of socioeconomic and BMI classes on BP.

Results. Obese subjects of upper socioeconomic class have higher blood pressure values. Strong significant differences in the mean values of systolic blood pressure (f-23.569; p<0.00001), diastolic blood pressure (f-22.470; p<0.00001) and mean arterial pressure (f-27.454; <0.00001) were observed in different BMI classes. Except for diastolic blood pressure (f-2.713; p <0.030) rest of BP indices did not differ significantly in different SES classes.

Conclusion. Obese subjects of upper socioeconomic class are prone for development of future hypertension. High BMI is significant risk factor for high BP, however socioeconomic class of the subject should also be considered as predisposing factor for high BP.

Keywords. blood pressure, BMI, obesity, socioeconomic class

Introduction

Economic transition in developing countries has resulted in changes in lifestyle and socioeconomic class of individuals.[1] Consequently the prevalence of overweight/obesity and hypertension is on rise and has reached to epidemic proportions.[1-3] It has replaced the more traditional causes of ill-health such as under nutrition and infectious diseases.[1,3,4] According to World Health Organization, cardiovascular diseases will be the largest cause of death and disability in India by 2020.[1,5,6]

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 12.06.2019 | Accepted: 16.06.2018
Publication date: June 2019

Soni A, Siddiqui NI, Wadhwani R. *Relative influence of body mass index and socioeconomic class on blood pressure levels and health*. Eur J Clin Exp Med. 2019;17(2):131–135. doi: 10.15584/ejcem.2019.2.4

BP is a complex entity which is influenced by many factors.[7] BMI and socioeconomic status (SES) are important determining factors for BP levels.[7,8] BMI has been extensively studied in the past epidemiological studies and was found to be positively associated with BP levels. Mean values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were reported higher in subjects with higher BMI.[9-11]

SES is an important psychosocial variable which has been observed to be associated with increased risk of stroke and hypertension.[15,16] The impact of change in SES on cardiovascular diseases and hypertension has been reported in the past studies however, literature on influence of SES and BP levels in normal healthy adults is still very limited.[2,12-14]

Aim

The purpose of this study is to ascertain influence of SES and BMI on blood pressure levels of a healthy adolescent population. This will help to reduce the future burden of cardiovascular disorders, resulting from change in socioeconomic class and obesity of the individual.

Material and methods

In this cross sectional study, three hundred (300) healthy adolescents with a mean age of 18.82 years (ranging from 18 to 25 years) were enrolled. After the subjects were instructed about the aims of the study, anthropometric measurements and BP recording were done as per the standard protocol.[17,18]

Weight was measured in standing position in light clothing without shoes to the nearest 0.1kg on a calibrated mechanical weighing scale (Krups India). Wall mounted stadiometer was used to measure height in erect posture to the nearest 0.1 cm. BMI was derived using standard formula BMI= Weight (kg)/ Height (m^2). Participants were classified into underweight (BMI < 18.5), normal weight (BMI from 18.5 to 24.9), over weight (BMI from 25 to 29.9) and obese (BMI >30) groups using WHO Asia Pacific guidelines.[19]

BP was recorded with a standardized mercury sphygmomanometer after five minutes of rest in a seated position with appropriate size of cuff applied to the right upper arm. Korotkoff phase-I was recorded as SBP and Korotkoff phase-V as DBP. Mean of last two readings with a time interval of one minute were used as final readings. Pulse Pressure (PP) was calculated by subtracting DBP from SBP. Mean Arterial Pressure (MAP) was calculated using the formula MAP = DBP+1/3 (PP). All the recordings were done at a fixed time (after 10:30 AM) by the investigator of the project without wearing white coat. For determination of socioeconomic class updated Kuppuswami scale was used.[20] Informed written consent and ethical clearance was sought prior to study.

Statistical analysis

Continuous variables were expressed as Mean and SD whereas categorical variables are expressed as number and percentages. Multiple regression analysis was used to find out significant predictors of BP. ANOVA and Tukey HSD post-hoc analysis was used to compare mean. The level of significance is fixed at $p < 0.05$. All the calculations were done on Med Calc (version 18.2.1) statistical software.

Results

The baseline characteristics of the study population are shown in the Table 1.

Table 1. Characteristics of study population

Parameter	Mean ± SD	95% CI
Weight (kg)	61.10 ±13.72	58.88 to 63.31
Height (cm)	166.0 ± 9.32	165.16 to 168.17
BMI (kg/m^2)	21.96 ± 4.28	21.27 to 21.27
SBP (mmHg)	123.0 ± 14.155	120.71 to125.28
DBP (mmHg)	74.70 ± 7.33	73.52 to75.88
MAP (mmHg)	89.709 ±8.91	88.696 to 90.721

On multiple regression analysis blood pressure indices (SBP, DBP and MAP) were significantly predicted by BMI, however socioeconomic class did not predicted blood pressure significantly (Table 2).

Mean values of SBP (135.75 ± 9.37 mmHg), DBP (80.62 ± 7.40mmHg) and MAP (99.00 ± 7.49 mmHg) were higher in obese group. Strong significant differences in the mean values of SBP ($f=23.569$; $p<0.00001$), DBP ($f=22.470$; $p<0.00001$) and MAP ($f=27.454$; <0.0000!) were observed in different BMI classes (Table 3). On Tukey HSD post–hoc test, highly significant intergroup differences were noted in different BMI classes except between overweight and obese class (Table 4).

As depicted in the Table 5, highest mean values of SBP, DBP and MAP were found in upper socioeconomic class (124.48 ± 18.67, 75.66±7.61 & 91.93 ± 10.74 respectively for SBP, DBP and MAP). On analysis of variance, except for DBP ($f=2.713$; $p= 0.030$) rest of BP indices did not differ significantly in different SES classes ($f=1.254$; $p = 0.288$ and $f=2.252$; $p=0.063$ respectively for SBP and MAP).

Prevalence of obesity and high blood pressure in the present study is 5.33% and 8.66% respectively. Highest number of subjects with elevated BP was found in upper socioeconomic class and overweight/obese category (Figure 1).

Table 2. Multiple regression analysis for prediction of BP indices

	Independent variables	Coefficient	Std. Error	$r_{partial}$	t	P
SBP (mmHg)	(Constant)	119.7405				
	BMI Class	4.0475	1.3615	0.1888	2.973	0.0033
	SES Class	-1.4590	0.9781	-0.09604	-1.492	0.1371
DBP (mmHg)	(Constant)	73.9170				
	BMI Class	1.8016	0.7268	0.1583	2.479	0.0139
	SES Class	-0.9762	0.5222	-0.1201	-1.869	0.0628
MAP (mmHg)	(Constant)	89.1907				
	BMI Class	2.5504	0.8636	0.1876	2.953	0.0035
	SES Class	-1.1370	0.6204	-0.1177	-1.833	0.0681

Table 3. Effect of BMI category on BP

	Under weight	Normal weight	Overweight	Obese	f- value	p-value
SBP (mmHg)	116.19±9.00	121.87±12.07	132.52±19.06	135.75±9.37	23.569	**<0.00001**
DBP (mmHg)	69.86±6.41	74.09±7.34	78.93±7.01	80.62±7.40	22.470	**<0.00001**
MAP (mmHg)	85.30±6.70	90.02±8.11	96.79±10.32	99.00±7.49	27.454	**<0.00001**

Table 4. Tukey HSD post-hoc test analysis of BP within BMI classes

BMI Class	SBP			DBP			MAP		
	Diff	95%CI	p	Diff	95%CI	p	Diff	95%CI	p
Under weight v/s Normal weight	5.6	1.43 to 9.92	**<0.001**	4.2	1.83 to 6.62	**0.003**	4.7	1.97 to 7.46	**<0.001**
Under weight v/s Over weight	16.3	5.76 to 12.37	**<0.001**	9.0	5.76to 12.37	**<0.0001**	11.4	7.70 to 15.2	**<0.001**
Normal weight v/s Obese	19.5	10.79 to 28.3	**<0.001**	10.7	5.82 to 15.69	**<0.0001**	13.7	8.03to 19.36	**<0.001**
Normal weight v/s Over weight	10.6	5.26 to 16.03	**<0.001**	4.8	1.80 to 7.87	**<0.0001**	6.7	3.28to 10.25	**<0.001**
Normal weight v/s Obese	13.8	5.43 to 22.32	**<0.001**	6.5	1.76 to 11.29	**<0.0001**	8.9	3.51 to 14.4	**<0.001**
Over weight v/s Obese	3.2	-6.13 to 12.59	0.80	1.6	-3.58 to 6.96	0.809	2.2	-3.84 to 8.2	0.78

Table 5. Effect of Socio-economic Class (SES) on Blood Pressure

B.P Indices	Upper Class	Upper middle	Lower Middle	Upper Lower	Lower	f- Value	p-value
SBP (mmHg)	124.48±18.67	122.01±11.75	120.00±11.5	119.64±10.9	119.5±8.38	1.25	0.288
DBP (mmHg)	75.66 ±7.61	73.80 ±7.29	72.24 ±8.39	72.07 ±7.22	68.5 ±4.12	2.71	0.030
MAP (mmHg)	91.93 ±10.74	89.87 ±7.94	88.16 ±8.87	87.92 ±7.81	85.50 ±5.1	2.25	0.063

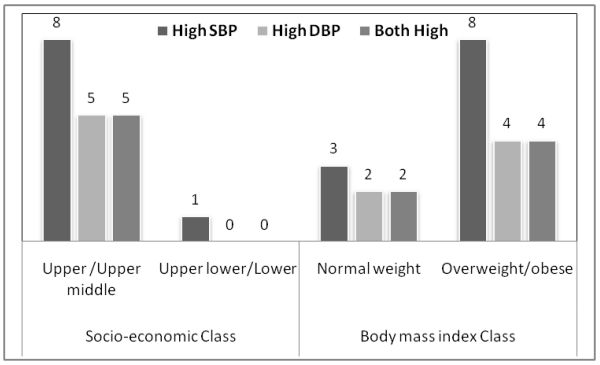

Fig. 1. Prevalence high BP within socio-economic and BMI classes

Discussion

There is paucity of data from developing countries on the prevalence of obesity and high blood pressure. Most of the reported data is from the developed countries which suggest higher prevalence of obesity and hypertension.[1-3]

The situation in the developing nations is changing rapidly due to rapid economic transitions. It has improved the socioeconomic status, reduces the physical activity and thus increases proneness for obesity and high BP.[3-5] It is because of this the prevalence of hypertension and obesity in the developing nations has reached epidemic proportions.[7-11]

In the present study SBP, DBP and MAP were significantly predicted by BMI. Mean values of SBP, DBP and MAP were significantly higher ($p<0.0001$) and differed across BMI categories . Subjects with elevated blood pressure (BP) levels were greater (25.08%) in over weight and obese group, indicating strong influence of BMI on BP. These findings are in agreement with previous studies which support that BMI is not merely a marker of obesity but it is causally associated with BP.[21-24] Obesity-associated rise in BP is a consequence of inadequate vasodilatation in presence of increased blood volume and cardiac output, which are natural consequences of an increase body mass.[25,26]

The impact of socioeconomic class (SES) on BP was studied in several epidemiologic studies with conflicting results.[4,11-14] In one of the past study high BP levels were observed in lower SES.[11] In another study negative correlation between SES and blood pressure (BP) was observed in normotensive subjects.[12] However, no association between SES and BP was observed in one of the previous study.[13] These variations might be due to differences in the populations and methodologies used to study this question. Most of the negative studies with regard to SES and BP were originated in underdeveloped countries suggesting that SES parameters influence BP differently in developed and developing societies.[27] In the present study mean SBP & DBP values were higher in upper SES. On statistical analysis only DBP has showed significant difference amongst the social classes. This is in agreement to the findings of past study.[4]

The mechanisms through which SES affect blood pressure are still unknown.[27] However It is suggested that the unfavorable metabolic and neuro-hormonal profile which is associated with a particular SES may contribute to the elevation of BP.[28] Furthermore, the relative impact of SES on BP is weak as compared to BMI.[27]

Total prevalence of obesity in the study was 5.33%, this is in agreement to the reported prevalence rates of obesity for India.[29] Highest numbers of subjects (8.66%) with elevated BP were found in over weight /obese and upper SES class respectively.

With the continued economic growth of the nation the percentage of people in the high socioeconomic group will increase further, this may lead to a higher prevalence of hypertension and cardiovascular disorders. Reduction in body weight early during the adolescent period particularly in high SES will be an important preventive measure to control this trend.[4]

Limitations

Small sample of a particular population group, single measurement of BP are the main limitations. Furthermore, causal relationships cannot be drawn because of cross-sectional nature of our study.

Conclusion

In the present study high BP were observed in overweight/obese and subjects in upper socioeconomic class, indicating their proneness for future hypertension. Therefore along with BMI, socioeconomic class of the subject should also be considered as a risk factor for high blood pressure.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.5

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 136–141

ORIGINAL PAPER

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Assessment of manual abilities in children with infantile cerebral palsy

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ABSTRACT

Introduction. Cerebral palsy (CP) is a problem presenting multiple issues and the prevalence of this condition is quite significant. CP risk factors are mainly observed in prematurely born children as well as those affected by complications around the time of birth or during the period of mother's pregnancy. Quite frequently CP is manifested by abnormal muscle tone, contractures and deformities, and consequently impaired fine and gross motor functions.

Aim. The study was designed to examine the level of hand function, i.e. fine motor skills and to investigate whether there is a correlation between development of fine motor and gross motor functions.

Material and methods. The study group included 80 children with infantile CP. In the group there were 24 cases with spastic diplegia, 36 with spastic hemiplegia, and 20 with bilateral hemiplegia. During the study the children performed Box and Blocks test, and their parents filled in Manual Ability Classification System (MACS) describing the level of fine motor function development in their children. The children were additionally asked to perform two motor tasks. The first one involved an attempt to assume position on all fours, and the other one checked the ability to assume and maintain standing position.

Results. The best scores in the conducted tests were found in children with CP taking the form of spastic diplegia, and the poorest scores in MACS, Box and Blocks test as well as in motor tasks assessing gross motor function were observed in children with bilateral hemiplegia.

Conclusion. The form of infantile CP affects the level of manual abilities. There is a correlation between the level of gross motor and fine motor functions development.

Keywords. fine motor function, gross motor function, infantile cerebral palsy, manual abilities

Introduction

According to definition, "Cerebral palsy (CP) describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to nonprogressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of CP are often accompanied by disturbances of sensation, perception, cognition, communication,

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 26.11.2018 | Accepted: 5.06.2019
Publication date: June 2019

Przysada G, Guzik A, Wolan-Nieroda A, Przybyło M, Drużbicki M, Mazur A. *Assessment of manual abilities in children with infantile cerebral palsy.* Eur J Clin Exp Med. 2019;17(2):136–141. doi: 10.15584/ejcem.2019.2.5

and behavior, by epilepsy, and by secondary musculoskeletal problems".[1] As a result functioning in daily life is also impaired. Problems are visible both in fine and gross motor skills, and are reflected by intellectual capacities, thought processes, deficits in mobility as well as abnormal muscle tone.[2-4]

Severity of the problems, patterns of motor involvement, and associated deficits, such as those in communication, and intellectual abilities, as well as epilepsy vary widely from subject to subject. In the most recent four decades the incidence remains at a constant level of 2-3 cases per 1,000 live births, despite the advancements in prenatal and perinatal care.[5,6]

Manual skills are reflected by the child's success in performing a specific activity. Manual tasks reflecting dexterity require engagement of fine and gross motor skills as well as motor coordination. Children with CP usually experience difficulties in performing manual operations, such as gripping, releasing or manipulation of objects, i.e. skills of key importance in many activities of daily living. Impaired functioning of upper limbs in children with CP is frequently associated with problems connected with motor control, active range of motion, grip strength and persistence of retained reflexes.[7]

Efficient hand function determines one's ability to cope with daily duties. It enables self-reliance and independence from other people. It is also a decisive factor for how much a person can learn. Without efficient hand function, we cannot independently take care of our needs, such as having meals and drinks, body care, dressing up, writing, or having fun. From the first stages of their life, children learn to explore the world using their hands.[8] Gradually acquired skills, such as hand grip or pressure applied or released consciously and intentionally, or adaptation of hand for varied functions enable people to become independent. Hence manual dexterity is a guarantee for effective functioning from conception to passing. Its gradual and progressive improvement is the foundation for the success in daily life. Even if there are deficits in fine motor skills, they can be compensated for, once hands take over the function, e.g. by moving with the help of special devices. Manual abilities are of critical importance for the quality of performance in daily life. Therefore it is necessary to highlight the role of hand therapy in children affected by CP.[9,10]

Aim

The main aim of the research was to assess manual abilities in children with CP; the secondary purpose of the study was to answer the following questions:

1. Does the level of hand function according to Manual Ability Classification System (MACS) test depend on the type of CP?
2. Is there a relationship between the score achieved on MACS scale and the number of blocks moved in Box and Blocks test?
3. Does the type of CP affect the score in Box and Blocks test?
4. Does the age of subjects affect the score in Box and Blocks test?
5. Is there a relationship between the score on MACS scale and the ability to assume the position on all fours and to stand (assume/maintain the position)?

Material and methods

The study group consisted of 80 children with CP, receiving rehabilitation treatment Special Care Educational Facility. In this group there were 24 children with diplegia spastica (spastic diplegia), 36 with hemiplegia spastica (spastic hemiplegia) and 20 with hemiplegia bilateralis (bilateral hemiplegia). The children were divided into three age groups. The first one comprised children aged 7-8 (15% 7-year-olds and 12.5% 8-year-olds). The second group consisted of children aged 9-10 (22.5% 9-year-olds and 12.5% 10-year-olds). The third group comprised children aged 11-12 (15% 11-year-olds and 22.5% 12-year-olds). The children participating in the study performed Box and Blocks test, where they were asked to move as many wooden blocks as possible from one box to another through a special partition, in course one minute, using respectively their left or their right hand. Higher scores in this test reflected better manual abilities, in terms of fine motor function. The Box and Block Test is an easy, feasible, valid, and reliable measurement for gross manual dexterity in children.[11,12] The parents were asked to specify the level of their children's manual skills with the use of Manual Ability Classification System (MACS), a scale designed to assess fine motor skills. MACS scale specified the child's ability to use objects during important every-day activities, for instance while playing, relaxing, eating or dressing up. Lower scores achieved by children with CP on MACS scale corresponds with their greater fine motor abilities, hence Level I on MACS scale represents the highest and Level V represents the poorest manual dexterity. As an addition, the children were asked to perform two motor tasks, i.e. to assume position on all fours, and to assume and maintain standing position.

All the subjects were informed about the purpose of the study and the procedure, and the children's parents or legal guardians provided written consent for their participation in the examinations. The study protocol was approved by the Bioethics Commission at the Faculty of Medicine.

Statistical analyses were computed with Statistica 10.0 software, respectively with the use of Pearson chi-square, Kruskal-Wallis and Spearman's rank correlation coefficient. Their choice resulted from a failure to meet basic assumptions of parametric tests, i.e. goodness of fit of the examined variables with normal distribution and homogeneity of variances. Goodness of fit with normal distribu-

tion was verified with Shapiro-Wilk test and homogeneity of variances with Levene's test. A test result where $p < 0.05$ was considered to be statistically significant.

Results

Description of the study participants' sex and the types of CP

The findings of the study showed that in the examined group of 80 children there were 44 (55%) boys and 36 (45%) girls. The children had been diagnosed with three types of CP; 30% of the subjects with spastic diplegia, 45% with spastic hemiplegia, and 25% with bilateral hemiplegia.

Analysis of the scores on the MACS scale

Analysis of the scores on the MACS scale showed that MACS Level I was represented by 30% of the subjects; Level II manual abilities were found in 47.5%, Level III in 12.5% and Level IV in the remaining 10% of the children. Assessment of scores on MACS scale in relation to age showed that the children aged 7-8 and 9-10 more frequently presented with Level II manual abilities, while the oldest children in the age range of 11-12 more often than the others reached MACS Level I. This correlation was not significant statistically - p=0.3710. Conversely, analysis of the scores on MACS scale relative to the type of CP showed highly significant correlation at the level of p=0.0000 (Table 1).

Table 1. MACS in relation to the type of infantile cerebral palsy

MACS		spastic diplegia	spastic hemiplegia	bilateral hemiplegia	Total
Level I	N	12	12	0	24
	%	50.0%	33.3%	0.0%	30.0%
Level II	N	12	22	4	38
	%	50.0%	61.1%	20.0%	47.5%
Level III	N	0	2	8	10
	%	0.0%	5.6%	40.0%	12.5%
Level IV	N	0	0	8	8
	%	0.0%	0.0%	40.0%	10.0%
Total	N	24	36	20	80
	%	30.0%	45.0%	25.0%	100.0%
p		x^2=54.44 p=0.0000***			

Analysis of the scores in the Box and Blocks test

Analysis of the scores achieved by the children in the Box and Blocks test, conducted separately for the boys and the girls, for the right and the left hand and relative to age range, showed that in the specific age groups the results for the right and the left hand were very similar. In the age group of the 7-8 year-olds, the girls achieved slightly higher scores, with mildly better results for the right hand; the boys, generally with poorer results, presented with higher scores for the left side. In the case of 9-10 year-old children, better results were recorded for the boys. In this age group, both the girls and the boys were more successful in the test examining the right hand. In the group of 11-12 year-old children, the girls coped with the task more effectively. Likewise, in this group both the girls and the boys scored higher in the right hand test. Generally, the best results were observed in the children aged 7-8, slightly poorer scores were achieved by the 11-12 year olds, and the lowest by those in the age group of 9-10 (Table 2).

Table 2. Score in Box and Blocks test for the right and the left hand in boys and in girls

Age [years]	boys				girls			
	Hand	mean	sd.	min-max	Hand	mean	sd.	min-max
7-8	R	24.0	11.1	10.0-38.0	R	29.4	12.6	10.0-42.0
	L	24.5	8.0	12.0-36.0	L	28.6	14.7	3.0-43.0
9-10	R	22.1	13.0	3.0-43.0	R	18.2	12.8	2.0-33.0
	L	19.5	9.2	2.0-34.0	L	13.2	11.5	0.0-27.0
11-12	R	23.0	11.6	7.0-42.0	R	25.7	14.7	3.0-41.0
	L	18.9	11.1	4.0-38.0	L	25.0	16.6	1.0-49.0

Statistical analysis based on Kruskal-Wallis test confirmed very high significance of the relationships, at the level of p<0.001***, between the number of blocks moved with both the right and the left hand and the type of CP in the examined children (Table 3). Hence, multiple comparison test was applied to find out which groups of children differed significantly in their performance in Box and Blocks trials. The differences in the number of moved blocks were found between the children with spastic diplegia and spastic hemiplegia versus the children with bilateral hemiplegia (successively for the right and the left hand p<0.001). No statistically significant differences were observed between the children with spastic diplegia and those with spastic hemiplegia (for the right hand p=0.5743 and for the left hand p=0.0854) – Table 4.

Table 3. Comparison of the scores for the right and the left hand acquired by children, relative to the type of infantile cerebral palsy

Right hand	(N)	mean	sd.	median	min-max
spastic diplegia	24	31.3	7.2	30.0	18.0-42.0
spastic hemiplegia	36	27.3	10.7	26.5	10.0-43.0
bilateral hemiplegia	20	7.4	4.5	7.0	2.0-15.0
p		H=42.19 p=0.0000***			
Left hand	(N)	mean	sd.	median	min-max
spastic diplegia	24	30.6	9.1	30.0	15.0-49.0
spastic hemiplegia	36	24.1	8.4	22.0	11.0-43.0
bilateral hemiplegia	20	5.1	4.4	3.5	0.0-12.0
p		H=48.17 p=0.0000***			

Table 4. Box and Blocks test – multiple group comparison

Box and Blocks test right hand	spastic diplegia	spastic hemiplegia	bilateral hemiplegia
spastic diplegia		0.5743	0.0000***
spastic hemiplegia	0.5743		0.0000***
bilateral hemiplegia	0.0000***	0.0000***	
Box and Blocks test left hand	**spastic diplegia**	**spastic hemiplegia**	**bilateral hemiplegia**
spastic diplegia		0.0854	0.0000***
spastic hemiplegia	0.0854		0.0000***
bilateral hemiplegia	0.0000***	0.0000***	

Associations between the Box and Blocks test and the MACS scale

Analysis with the use of Kruskal-Wallis test confirmed very high statistical significance at $p<0.001^{***}$ ($p=0.0000$ for the right hand and $p=0.0003$ for the left hand), of the relationship between the number of blocks moved with both the right and the left hand, versus the children's scores on the MACS scale. Given this, multiple comparisons test was applied to determine which groups of children differed significantly in their performance during Box and Blocks trials. Differences in the numbers of transferred blocks were found between the children with MACS Level I and II fine motor skills and the children with Level III and IV fine motor skills. These results were related to both the right and the left hand (Table 5). The best scores in Box and Blocks test were achieved by the children classified at MACS Level I. The poorest results in the blocks test were scored by children located at Level IV on MACS scale.

Table 5. Box and Blocks test – multiple group comparison

Box and Blocks test right hand	Level I	Level II	Level III	Level IV
Level I		0.0267*	0.0000***	0.0000***
Level II	0.0267*		0.0016**	0.0003***
Level III	0.0000***	0.0016**		1.0000
Level IV	0.0000***	0.0003***	1.0000	
Box and Blocks test left hand	**Level I**	**Level II**	**Level III**	**Level IV**
Level I		0.0603	0.0000***	0.0000***
Level II	0.0603		0.0225*	0.0009***
Level III	0.0000***	0.0225*		1.0000
Level IV	0.0000***	0.0009***	1.0000	

Relationships between the tests assessing fine and gross motor skills

Analyses were also performed to investigate the relationships between the tests assessing fine and gross motor skills in children with CP. Analysis based on Spearman's rank correlation test confirmed statistically very high correlation, at $p<0.001^{***}$, between fine motor skills in the children (MACS), and gross motor skills as reflected by the specific elements related to the ability to assume all-fours position and to assume and maintain standing position without assistance. The observed values of correlation were very strong. They achieved negative values at the level of $|R=-0.7|$. The negative orientation represents an increase in the value of one variable coinciding with a decrease in the value of the other variable. The higher the MACS Level achieved by the children, the more often they were unable to perform the task. Hence, poorer fine motor abilities in the children coincided with poorer gross motor skills – as reflected by the inability to perform the consecutive motor tasks and vice versa, the better the fine motor skills, the more successful the children were in tasks involving gross motor function.

Discussion

A number of tools designed to verify the level of fine motor skills in children affected by CP have emerged in recent years. These include the Manual Ability Classification System.[13-19] Jeevanantham et al. suggest that the MACS could be considered as a standard classification for children with cerebral palsy on the basis of manual abilities, and can be reliably used for children between 4 and 18 years.[20] In the current study the MACS was applied to assess children ranging in age from 7 to 12 years. Our findings show that in the group of children with spastic diplegia 50% achieved Level I manual abilities, which is evidence of well-developed motor function, and the remaining 50% of the subjects in this group achieved MACS Level II. In the group of children with spastic hemiplegia, 33.3% achieved Level I manual abilities, 61.1% Level II and only 5.6% of the subjects were classified at Level III on MACS scale. As for the next type of infantile CP, i.e. bilateral hemiplegia, none of the affected children achieved MACS Level I, while 40% of the children were classified at both Level III and Level IV of the scale.

Similar results were reported by Michalska et al., the poorest results were obtained by children with bilateral hemiplegia; as many as 60% of the study group were classified at Level IV and V, according to MACS scale.[21] According to a study carried out by Department of Hand Surgery in Stockholm, problems related to daily activities involving upper limbs were encountered by 80% of the children with spastic hemiplegia and 68% of the children with spastic quadriplegia.[22] A study by Mazanek suggests that poor experience related to gross motor abilities contributes to limitations in fine motor skills. Children affected by CP taking the form of bilateral hemiplegia achieve the poorest results related to both fine and gross motor functions. In MACS they are also classified at the two lowest levels existing in the scale – i.e. Level IV or V. [23] When it comes to the scores in Box and Blocks test it is possible to notice the follow-

ing relationships. Children with spastic diplegia on average scored 31.3 points in the trial with the right hand, and 30.6 points with the left hand. Again, the poorest results were found in children with bilateral hemiplegia who scored only 7.4 points with the right and 5.1 points with the left hand. Our study also shows that age does not significantly affect scores obtained in Box and Blocks test. Hence, it was observed that the results for the right and the left hand were similar in all age groups. Statistically significant differences in the number of transferred blocks were recorded between children with spastic diplegia and spastic hemiplegia, versus children with bilateral hemiplegia (successively for the right and the left hand $p<0.001^{***}$). No statistically significant differences were observed between the children with spastic diplegia and spastic hemiplegia (for the right hand $p=0.5743$ and for the left hand $p=0.0854$).

Our study has shown that in the case of right hand dominance, both the girls and the boys achieve higher scores in Box and Blocks test for the right hand. According to a study by Mathiowetz et al., children with the dominant right hand achieved better results than children with the dominant left hand. Additionally, lower precision was observed when blocks were moved with the left hand. That study also showed that with the growing age of the children their scores in Box and Blocks test were increasing.[24]

The present study has also established a relationship between the level of gross and fine motor functions development. The motor tasks examined the ability to assume all-fours position and to assume and maintain the standing position. Better classification on MACS scale corresponded with the children's greater gross motor skills.

The current findings also show a correlation between the level achieved on MACS scale and the number of blocks transferred in Box and Blocks test. A higher level in MACS classification corresponded with a smaller number of blocks transferred in Box and Blocks test. Similar results were reported by Golubović et al. who in their study assessed manual ability and manual dexterity in children with cerebral palsy. The study involved 30 children with cerebral palsy. In order to assess gross manual dexterity the Box and Block Test was used. Manual ability was assessed according to MACS scale. The researchers established there was a relationship between the level of manual ability impairment and performance on manual dexterity tasks. Participants at MACS level IV transferred the smallest number of blocks ($p<0.01$). All manual skills were more impaired in the non-dominant hand compared to the dominant hand but there were no statistically significant differences ($p=0.06$).[25] Similarly, Öhrvall et al. and Araneda et al. also demonstrated a strong association between the MACS and the Box and Block test ($p<0.05$). They observed significant differences in the performance of the children with CP between the various MACS levels and the Box and Block test ($p < 0.001$).[26,27]

These findings suggest that the MACS and the Box and Block test provide reliable information on manual abilities in children with CP. The data identified using these tools represent comprehensive overview of the child's capacities, thereby facilitating the processes of designing a treatment plan and monitoring of therapies administered. The clinical applicability of the main results is also reflected by the correlation between the level achieved on the MACS scale and the number of blocks transferred in the Box and Blocks test, since this shows that the tools can be used interchangeably in assessment of children with CP. What is more, it could theoretically be assumed that children with spastic diplegia, which mainly affects gait function, would present with manual dexterity similar to that in healthy children. However, the scores in Box and Blocks Test acquired in the present study by boys and girls representing three age groups were examined with reference to the norms for children [12,13], and it was found that the scores acquired by the subjects with spastic diplegia were nearly two times lower. These findings suggest that manual dexterity in these patients significantly differs from the norm. The limitation of the study was the fact that there was no control group consisting of healthy children; these will be included in further research.

Conclusions

The level of manual dexterity depends on the type of infantile CP. The children with spastic diplegic CP presented with the best manual skills. The poorest functional hand performance was observed in children with bilateral hemiplegia.

There is a relationship between the level achieved on MACS scale and the number of blocks transferred in Box and Blocks test. The lower the level acquired by the child in MACS classification, the greater the number of blocks moved by him/her, and the higher the level in MACS classification, the fewer the number of blocks successfully transferred in the task.

Type of infantile CP significantly impacts the score obtained in Box and Block test. The children with spastic diplegia acquired the highest scores. Similar, yet slightly lower scores were obtained by children with spastic hemiplegic CP. The poorest results were acquired by children affected by bilateral hemiplegia.

Moreover, age of children with CP does not significantly affect the scores in Box and Blocks test.

There is a correlation between the identified MACS level and selected gross motor skills. The study has shown that the ability to assume all-fours position and to assume/maintain standing position is significantly related to the quality of fine motor function development.

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© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.6

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 142–145

REVIEW PAPER

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Imaging methods of early detection and screening for breast cancer. A review

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ABSTRACT

Introduction. Breast cancer is the most frequent neoplasm among women. That is the reason why scientists all over the world are attempting to improve early detection methods of this particular malignancy.

Aim. The most common and most widely used examination methods for screening for and detecting breast cancer is presented herein.

Material and methods. This review was performed according to systematic literature search of three major bibliographic databases.

Results. Available data suggest that incidence and mortality in high-resource countries has been declining whereas incidence and mortality in low-resource countries has been increasing.

Conclusion. The role of a physician is to select the most suitable one for each patient in order to obtain the best result. No matter the method however, between 2005 and 2011, the 5-year relative survival was found to be 89%. This is thought to be due to both the increase in utilization of population-wide screening, as well as advances in treatment.

Keywords. breast cancer, breast MRI, early detection, screening

Introduction

The most common malignancy in women is breast cancer.[1-30] According to the Polish National Cancer Register, it is also second most common cause of cancer death.[1-30] This is probably because risk factors and access to early detection methods and treatment differ in those regions.[1-30] Risk factors for breast cancer include increasing age, race, menarche history, breast character-

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 7.11.2018 | Accepted: 29.11.2018
Publication date: June 2019

Gustalik J, Bartusik-Aebisher D, Aebisher D, Kaznowska E. *Imaging methods of early detection and screening for breast cancer. A review.* Eur J Clin Exp Med. 2019;17(2):142–145. doi: 10.15584/ejcem.2019.2.6

istics, reproductive patterns, hormone use, alcohol use, tobacco use, diet, physical activity, and body habitus.[1-30] Mutations in the BRCA 1 and BRCA 2 tumor suppressor genes are significantly associated with the development of breast and ovarian cancer by the age of 70.[1-30] Survival depends on both stage and molecular subtype.[1-30] As there are few signs and symptoms early on, early detection is an important strategy to improve outcomes.[1-30] It is not a secret that detecting cancer at an early stage or even precursor lesions provide patients with a better chance of survival. That is why it is so important to constantly evaluate accessible early detection methods and to search for new ones.

Early detection and screening methods

Eighty-one percent of breast cancers are diagnosed among women ages 50 years and older, and 89% of breast cancer deaths occur in this age group. The median age at diagnosis for women with breast cancer is 62 years.[30-31]

Breast self-examination

For a number of years breast self-examination was promoted as the simplest method for breast cancer detection.[32-38] However, the recent studies show that it does not reduce the breast cancer mortality and is not effective to diagnose cancer at an earlier stage.[39] It has also been revealed that it increases the number of unnecessary interventions in women due to false-positive results of this examination method.[3,38]

Ultrasonography

Conventional ultrasound for breast screening is efficient and relatively easy to perform; however, it lacks systematic recording and localization.[40] Ultrasound examination is recommended for breast cancer screening in young women whilst mammography is recommended for older female patients. This is due to high density of fibrogladular tissue which is better visualized in ultrasound.[2,7]

Mammography

Mammography is the only imaging modality to have been shown to reduce mortality rate in asymptomatic age-appropriate women.[28] However, sensitivity of this examination method can be limited in dense breast tissue especially in younger patients because fibroglandular tissue reduces visibility of abnormalities.[2,28] Another aspect worth mentioning is the growth pattern – if the tumor does not produce a mass it is very difficult to detect in mammography.[7,28]

Digital breast tomosynthesis (DBT)

Digital breast tomosynthesis is relatively new method of breast imaging. In this examination, radiologists reconstruct a 3D image from multiple low-dose 2D x-ray source projection images. Obtained data allow them to evaluate breast tissue in very thin slices (e.g. 1 mm). [5,8,28]

Breast magnetic resonance imaging (MRI)

Breast MRI is a very useful tool not only for detection and characterization of breast cancer but also for describing the extent of the tumor and evaluation of the treatment response.[10,28]

Reported sensitivity of breast MRI in detection of invasive breast cancer has approached 100% in several series.[28,29] This data in one of the reasons why breast MRI is important in preoperative staging.[28, 29] The limitation of breast MRI is low-to-moderate specificity ranging from 37-97%.[28-36]

Comparison of the aforementioned methods of early detection of breast cancer

There are many studies comparing usefulness of different methods of early detection of breast cancer. Many factors contribute to these results. It is believed that breast self-examination is the least useful method of all.[3,38] It also is the reason for unnecessary medical interventions in female patients.[3,38]

Mammography is the gold standard in breast cancer detection.[41] It provides a good quality image with reduced radiation dose and can detect breast carcinoma in its earlier stages, resulting in good prognosis and improved patient survival.[41] Obese women are the ones with the highest sensitivity of screening mammography, while the specificity of screening remained stable across weight groups.[21]

The exclusive use of quality-assured breast MRI allows the early detection of breast cancer with a high sensitivity and specificity.[9] Additionally, breast MRI is a reliable problem-solving method for excluding malignancy that cannot be confirmed by conventional imaging. In such cases, additional findings from MRI may help identify new cancers that cannot be detected with conventional methods. However, it has moderately low specificity which may cause unnecessary biopsies, follow-ups, and anxiety to patients.[12]

Overdiagnosis

There are many studies concentrating on the rate of overdiagnosis in female patients with breast cancer.[15-41] This unfortunately leads to unnecessary medical procedures that could otherwise be avoided. The most plausible estimates of overdiagnosis range from 1% to 10%. Substantially higher estimates of overdiagnosis reported in the literature are due to the lack of adjustment for breast cancer risk and/or lead time.[24] MRI yielded the highest performance even though the unexpected specificity may lead to over-diagnosis, and ultrasonography is slightly better than mammography.

Conclusion

Many different imaging methods of early detection and screening for breast cancer are used throughout the world. Indications to application of each method vary. The role of a physician is to select the most suitable one for each patient in order to obtain the best result. No matter the method however, between 2005 and 2011, the 5-year relative survival was found to be 89%. This is thought to be due to both the increase in utilization of population-wide screening, as well as advances in treatment.

Acknowledgments

Dorota Bartusik-Aebisher acknowledges support from the National Center of Science NCN (New drug delivery systems-MRI study, Grant OPUS-13 number 2017/25/B/ST4/02481).

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.7

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 146–152

REVIEW PAPER

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Use of whole-body vibration as osteoporosis treatment in postmenopausal women: a systematic review

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ABSTRACT

Introduction. The use of whole-body vibration (WBV) has increased in the therapeutic field for patients with osteoporosis, however, there is still some controversy about its real effects.

Aim. to perform a systematic review on the use of WBV for improving bone mineral density and effects of osteoporosis in postmenopausal women.

Material and methods. the search was conducted by two researchers in the MEDLINE/PubMED and SciELO databases. It was included in the study clinical trials that dealt with the influence of vibration platform treatment on osteoporosis in the Portuguese and English languages published since 2006.

Results. Ten selected clinical trials were found in a total of 405 articles. There are heterogeneous results owing to the divergences of the study. Six articles presented benefits of treatment with WBV in bone parameters, one article with changes in balance and muscle strength and three with no effects after treatment.

Conclusion. It is concluded that the use of WBV was presented as an option in the treatment of osteoporosis, however, studies using homogeneous methodologies are needed to compare the actual benefits of using them.

Keywords. bone and bones, exercise, osteoporosis

Introduction

Osteoporosis results from an imbalance between bone formation and resorption, affecting patients at different ages and altering normal bone architecture.[1,2] Aging increases the incidence of fractures associated with this disease, particularly in postmenopausal women, and the term postmenopausal osteoporosis is used when estrogen deficiency exacerbates demineralization of the bone architecture.[3–6]

This process occurs due to estrogen acting as a bone protector, decreasing bone resorption by increasing the

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 23.05.2019 | Accepted: 10.06.2019
Publication date: June 2019

Peretti AL, Tozeto Ciqueleiro R, Forner Flores LJ, Flor Bertolini GR. *Use of whole-body vibration as osteoporosis treatment in postmenopausal women: a systematic review*. Eur J Clin Exp Med. 2019;17(2):146–152. doi: 10.15584/ejcem.2019.2.7

expression of osteoprotegerin, secreted by osteoblasts and decreasing, among others, the expression of the nuclear transcription factor kappa B ligand (RANKL) in osteoclasts. Although maintenance of bone tissue with altered concentrations is maintained, this estrogen deficiency increases the levels of proinflammatory cytokines and tumor necrosis factor, stimulating osteoclastogenesis with increased expression of the activating receptor of RANKL.[7–9]

Fractures that occur due to bone fragility are a serious public health problem worldwide, since they have large proportions on mortality, morbidity and quality of life.[10] Regular exercise may be in charge of increasing and maintaining bone mineral density (BMD), improving balance, and reducing the risk of fractures and falls.[3,11,12] However, although physical exercise is beneficial, physical exercises may be contraindicated or difficult to perform in elderly individuals, thus increasing the likelihood of developing chronic diseases and their comorbidities.[1,3] For this reason, new methods of intervention for the increase in BMD have been increasing recently and among them, training in vibratory platform or whole-body vibration (WBV) is cited as a way to increase BMD, treat osteoarthritis and improve physical fitness, both in human and animal studies, although there are still several controversies about its effects.[12–19]

On these platforms, individuals receive mechanical stimuli that causes vibration of the entire body.[14,20,21] Its benefits in the bone tissue can be explained by the piezoelectric effect, because after a mechanical tension there is the generation of electric potential along the tissue, with negative charges generated on the bone surface and consequently the flow of positive ions to the extracellular medium after activation of ionic channels, stimulating the mechanotransduction, that is, osteocytes to generate biochemical responses and induce the formation of bone mass and suppress their resorption.[22,23] WBV is also capable of promoting muscle strength gains by potentiation muscle contraction after proprioceptive activation. It is also important to emphasize that the strengthening of periarticular muscles control the weight loss in the involved joints, reducing shock and overloads, which would also affect the skeletal system beneficially.[24,25]CINAHL, Embase, Scopus, PEDro, and Science citation index for research articles published prior to March 2015 using the keywords whole body vibration, vibration training, strength and vibratory exercise in combination with the Medical Subject Heading 'Osteoarthritis knee'. Study selection: This meta-analysis was limited to randomized controlled trials published in the English language. Data extraction: The quality of the selected studies was assessed by two independent evaluators using the PEDro scale and criteria given by the International Society of Musculoskeletal and Neuronal Interactions (ISMNI).

However, there are contraindications to its use, such as in diabetic individuals with neuromuscular diseases, severe heart disease, stroke, implant, bypass, stent; furthermore there is disagreement about the real effects of the use of WBV in the bone architecture and improvement of osteoporosis in postmenopausal women, as well as with respect to the parameters used, and the tissue responses are dependent on these factors, with the literature pointing out that its use is more effective when not used for strenuous periods and with frequencies between 30-60Hz.[3,13,26,27]Grupo Aula Medica S.A. All rightsreserved. Objective: The aim of this study was to examine the effect of 8 months of whole-body vibration training on bone mass in octogenarian women. Method: Thirty-seven women (aged 82.4 [SD=5.7] years).

Aim

In this way, this study aimed to carry out a systematic review of the literature on the effects of WBV on bone mineral density and whether it is possible to attenuate the effects of osteoporosis in postmenopausal women.

Description of the subject literature

This systematic review was carried out according to the criteria and recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) methodology. We used the National Library of Medicine (MEDLINE / PubMED) and the Scientific Electronic Library Online (SciELO) electronic databases, investigating articles related to the proposed topic, searched in the period of May and June of 2018. The searches were performed with the terms "osteoporosis", "vibratory platform", "whole-body vibration", "bone", articulating also the words with "and", thus forming combinations.

We included in the study randomized clinical trials that had as an object of study the influence of the treatment of WBV on osteoporosis, published since 2006, with articles available in full online in Portuguese and English. Studies that did not fit these criteria and did not have menopausal women as participants were not included in the study. The analysis of the selected articles was done by two authors, if there was disagreement, a third author would be responsible for inserting the article or not in the review. The sequence of steps to select the articles was first made by the identification and selection of articles, followed by the eligibility in which the inclusion and exclusion criteria were implemented, and finally the inclusion of the articles chosen. For the included articles, the Physiotherapy Evidence Database (PEDro) scale was used to measure the methodological quality of the studies, also applied by two evaluators.

After searching the databases, a total of 405 scientific articles were found, 360 of which were found in MEDLINE / PubMED and 45 in SciELO. However, some

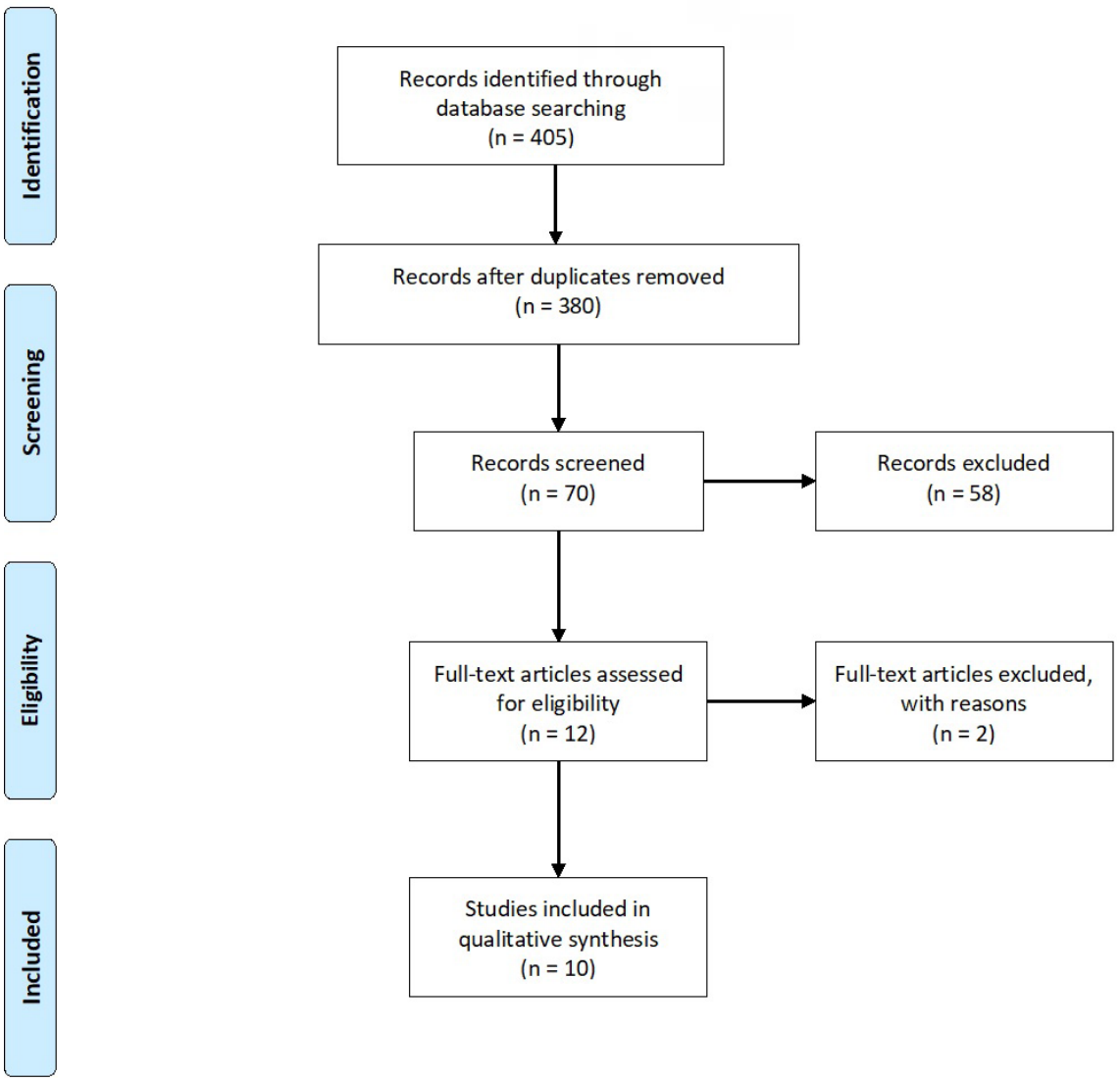

Fig. 1. Flowchart (based on PRISMA 2009) to identify the articles included in the study

articles were duplicated in the databases, thus totaling 380 articles initially. Following this step, a careful analysis of the articles occurred, by means of title and abstract readings, and later for pre-selected articles with full text reading, reaching an end of 10 articles selected for this systematic review. A flowchart was elaborated (Figure 1), to visualize the selection stages of the articles.

The methodological quality assessed by the PEDro scale resulted in an average of 8.2 ± 1.7. Higher scores do not necessarily imply clinical evidence, but they assure that the treatment used was adequate, with studies varying from 6 to 10 points on the scale.

The results showed that the periods can vary from 6 to 12 months of treatment, and the use of WBV may be involved in exercise, as well as vitamin D and calcium supplementation. In the articles studied, 6 suggest beneficial effects of intervention on bone, muscle and balance parameters.[28–31] One study had no effect on BMD, however, it decreased the frequency of falls.[32]the multi-purpose exercise training was effective to increase lumbar BMD but added WBV did not enhance this effect. However, falls were lowest in the exercise program combined with WBV. Introduction: WBV is a new approach to reduce the risk of osteoporotic fractures. In the \"Erlangen Longitudinal Vibration Study\" (ELVIS). Three other studies did not present significant consequences for the parameters evaluated (Table 1).[33–37]

Analysis of the literature

With the mechanical load it is possible to stimulate bone formation and maintenance of muscle mass, and body vibration is a proposal that is growing among the modalities of physical exercise.[38,39]selective effects of different frequency and acceleration magnitude modalities on musculoskeletal responses need to be better defined. Our aim was to investigate the bone effects of different

Table 1. Description of the articles included and analyzed, with presentation of the authors, participants, main interventions and evaluation forms, results found and a note attributed by the PEDro scale

Author / year	Participants	Intervention	Evaluation	Outcome	PEDro Scale
Gusi et al., 2006	Groups Walk Exercise (n=14) and WBV (n=14)	8 months, 3x/week. Performed walking exercise (60min) or WBV (1-30min, 12.6Hz)	BMD femur and lumbar spine and balance	WBV group improved femur BMD and balance	7
Beck et al., 2010	Groups Control (n=15), low (n=15) and high intensity WBV (n=17)	8 months. Control group without intervention, WBV 2x/week with low (15 min, 30Hz, 0.3G) and high (2x3 min, 12.5Hz, 1G) intensity	Bone mineral content, lumbar spine BMD, femur, forearm, muscle strength and balance tests	WBV improved the parameters, without differences between high and low frequency	6
Slatkovska et al., 2011	Groups Control (n=67), low (n=68) and high WBV (n=67) frequency	12 months. Control group without intervention. Daily use of WBV (20min, 0.3G) of low (30Hz) and high (90Hz) frequency + supplementation of vitamin D and calcium for the three groups	Bone volume and thickness of the tibia and radius, femur BMD and lumbar spine	No difference between groups	10
Verschueren et al., 2011	Control Groups: Vitamin D supplementation with low (n=28) and high (n=29) dose; PV: Vitamin D low (n=28) and high (n=26) dose	6 months. Control groups without intervention and WBV groups (1-12 min, 30-40Hz, 1.6-22G), frequency 3x/week, associating static and dynamic exercises in the platform	Hip BMD and isometric and dynamic strength, muscle mass of knee extensors	Vibratory platform did not increase the parameters in comparison to the use of vitamin D, also not having difference between the doses	7
Von Stengel et al., 2011	Groups Low-intensity exercise (n=33), Training Rotational Vibration (n=29) and Vertical (n=34)	12 months, 3x/week. Exercise Group with 2x10 repetitions of low intensity exercises, rotational WBV of 12.5Hz, 0.8G, vertical PV of 35Hz, 0.8G, both associated with dynamic exercises during 15min	BMD lumbar spine and femur, muscle strength	Improves BMD and strength for both groups platform	10
Von Stengel et al., 2011	Groups Low intensity control (n=48), Training (n=47) and Training + WBV (n=46)	18 months, 2x/week Control group with light exercises, Group Training with conventional aerobic exercises, balance and muscle strength during 60min and when associated with WBV, used 25-35Hz for 6min	BMD hip and lumbar and frequency of falls	The use of WBV does not improve the effects that the training already promotes, but improves the frequency of falls	10
Lai et al., 2013	Control groups (n=14) and WBV (n=14)	6 months. Control group without intervention and WBV (30Hz, 3.2G), 3x/week, 5min	Lumbar spine BMD	Significant improvement for the WBV group	9
Stolzenberg et al., 2013	Groups Balance training (n=31) and WBV (n=26)	9 months, 2x/week. 15min progressive balance exercises and 4min PV (22-24Hz, 2-4mm, 3.9G-10.3G) with different postures	Peripheral CT of the tibia, fibula, radius and ulna	Improvement in both groups	7
Liphardt et al., 2015	Control groups (n=20) and WBV (n=22)	12 months. Control group without intervention, WBV (20Hz, 3-4mm) 2-3x/week, 10min	BMD, balance, jump performance and maximum voluntary contraction of flexors and knee extensors	There was no difference between the groups for the parameters evaluated	6
Shanb et al., 2016	Pharmacological treatement (PT) (n=25); Magnetic therapy+PT (n=30) and WBV+PT (n=30)	4 months. WBV (20-24Hz) 2/week, 55min	BMD, venous blood (calcium and vitamin D)	PV and magnetic therapy were superior to the medication only group, yet with no differences between them	10

Legend: n - number of participants, x - times, min - minutes, Hz - hertz, G - acceleration of gravity, mm - millimeters (displacement), WBV – whole-body vibration platform, BMD - bone mineral density, CT - computadorized tomography.

vibration frequencies at constant g level. Vertical WBV was delivered at 0.7. g (Peak acceleration In this review, five clinical trials with positive effects for the treatment of osteoporosis were found. In the study by Lai et al. a WBV group (30Hz frequency, magnitude 3.2G) and a control group were assessed. After treatment, the WBV group increased lumbar spine BMD.[28] The authors suggest that this occurred because of the training using high frequency and high magnitude and for assessing the lumbar spine, the neutral orthostatic position on the platform favors the direct transmission of the vibration to the spine, in addition to the duration of each session being five minutes, without provoking deleterious effects, and being an exercise of easy adhesion.

Stolzenberg et al. compared the WBV Group (24-26Hz frequency progression and 2-4mm amplitude) with a Balance Training (BT) group after performing resistance exercises, finding an increase in BMD in the tibia in both groups, with no significant difference between them. The authors point out that a significant improvement between the groups could be perceived through more modern measurements, such as Dual Emission X-ray, and suggested that these results appear in shorter treatment periods with vibration training with frequencies and longer durations for a better stimulus. In addition, there was a difference for the BT Group in the parameters that evaluated the radius and the ulna, which is justified by the fact that this group also performed exercises for upper limbs, which the WBV Group did not do so it is necessary to perform new studies that analyze the effect of body vibration also on upper limbs.[29]

In the work of Von Stengel et al. two types of vibration were evaluated: vertical (35 Hz, 1.7 mm amplitude) and rotation (12.5 Hz, 12 mm amplitude), those associated with muscle strengthening exercises, compared to Control group with low intensity exercises.[30] Two groups of vibration improved BMD of the lumbar spine, but did not affect BMD of the femur. However, the vertical group presented results in the non-significant borderline, different from the rotation group, possibly explained by the given stimulus (rotation plate frequency) being twice as strong in the spine as in the femur region. In addition, individuals positioned themselves on the platform with slight flexion of the lower limb joints and the vibration transmission is dependent on this flexion, being greater in the vertical position, with the semi-squat position reducing acceleration up to ten times in the hip, explaining this possible result and corroborating that found in the article by Lai et al..[28] In addition, the gain of muscle strength in both groups of vibration can be explained by the concomitant use of exercise and the position of semi-flexion, different from other studies that do not associate the exercise.

In another study, with a population with the same characteristics as the previous one, Von Stengel et al. sought to evaluate whether VF (frequency 25-35 Hz and 1.7 mm amplitude) would enhance the effects of physical training on BMD and the frequency of falls in women. Physical training addressed aerobic, functional and balance exercises. The results suggest that both training and training groups associated had an increase in BMD in the lumbar spine when compared to low intensity control. However, the number of falls decreased significantly only in the group that associated with the training of the WBV. Thus, it is evident the benefit that physical exercises can promote in bone tissue due to mechanical loading and weight loss, but, although it does not directly contribute in this study to these alterations, possibly due to the time and period used, WBV reduced the number of falls, being this important factor for the risk of fractures.[32]the multi-purpose exercise training was effective to increase lumbar BMD but added WBV did not enhance this effect. However, falls were lowest in the exercise program combined with WBV. Introduction: WBV is a new approach to reduce the risk of osteoporotic fractures. In the \"Erlangen Longitudinal Vibration Study\" (ELVIS).

In the study by Gusi et al. a 55-minute walk exercise group per session (3x week) and another exercise group with WBV of 12.6 Hz and 3mm amplitude were compared for 8 months. The WBV Group was more effective in improving balance and BMD, only in the femoral neck and not in the lumbar spine, probably also explained by the position of the subjects on the apparatus, with a knee flexion of 60º, which affects the vibration to the spine. The authors suggest that the platform stimulus is different from the day-to-day walking stimulus, causing adaptations in the bone tissue, in addition to the low magnitude and high frequency used. Another possible result could be found with resistance exercises, which were not used in the study.[33]increasing the lateral accelerations. A few studies have shown recently the effectiveness of the up-and-down plate for increasing Bone Mineral Density (BMD).

Beck and Norling aimed to determine the effects of high (12.5 Hz, 1G) and low intensity (30Hz, 0.3G) WBV twice weekly during a period of eight months on the risk of hip fracture in women post menopause. They observed that the use of low intensity WBV helped in the slight but not significant mass gain in the femoral neck region and in both intensities the use of the platform assisted in the maintenance of the bone mass in the regions of the neck of the femur and spine, when compared to the control group.[34]

In the study by Liphardt et al., the group that associated with WBV had no improvement in BMD, area and mineral resistance, in addition to parameters of muscle strength and balance. They suggest that to potentiate a bone change, it would be a necessary concomitant to the training of vibration, the resistance training, which was

not associated with the study. There was only difference in the parameters of muscular strength in the first four months, being justified by the action of the WBV in promoting stimuli to the sensory system, provoking a period of adaptation, however, this stimulus is not able to cause physiological changes measurable since after this period the values were the same as those found in the control.[35] According to Lai et al., these adverse effects can be justified due to exposure of great intensity, magnitude or long duration, which also affects blood vessels and peripheral nerves.[28]

In the study by Slatvoska et al., the therapy of high (90Hz, 0.3g magnitude) and low (30Hz, 0.3G) frequency was unable to change the BMD and bone structure of postmenopausal women who received calcium and vitamin D. The authors justify these results because they obtained median adherence ranging from 65-79% and because the treatment was administered at home and without supervision, which makes it impossible to know if the participants performed the activities correctly and following recommended posture and instructions.[36] Verschueren et al. associated WBV with vitamin D supplementation in institutionalized elderly women. There were no differences between the groups analyzed. This situation can be explained by the lack of a control group without vitamin D supplementation associated with physical exercise, and a control group without PV activities and supplementation, which makes it impossible to verify the real influence of the use of PV in the elderly in an isolated way.[37] Shanb et al. compared the PV associated with drug therapy with magnetic therapy, and after 4 weeks of therapy, they observed that both groups were superior to the BMD results compared to the only medication group, but with no differences between them.[31] The use of WBV in the treatment of osteoporosis can be used to minimize or prevent the changes caused by this disease in the body, however, there are no standards defined in the literature regarding the frequency, duration of the intervention and the period to be used. Clinical trials present heterogeneous results due to the divergences used in the methodology, however, in short, the platform has positive effects in the treatment of osteoporosis when used at high frequency and associated with physical exercises, but the low frequency can also help positively the parathyroid response and thus, targeting positive results in bone tissue.[40]

Conclusion

We concluded that the use of WBV is presented as an option in the treatment of osteoporosis, however, studies using homogeneous methodologies are needed to compare the actual benefits of using them.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.8

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 153–156

REVIEW PAPER

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Neoadjuvant therapy in breast cancer – objectives and tasks

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ABSTRACT

Introduction. Neoadiuvant therapy (NCT) in the treatment of breast cancer is employed for patients with early stage disease or with inoperable disease. NCT can decrease the tumor volume. It can facilitate breast conservation therapy. Response to NCT is a strong predictor of outcome breast cancer (BC). Direct target therapies has markedly improved the result of treatment BC.
Aim. Therapy for breast cancer continues to improve. The importance of tumor burden on local control rates will be in the future.
Material and methods. This analysis was performed using a systematic literature search.
Results. The latest scientific reports give hope for greater safety and a better life for patients based on optimized and effective therapy.
Conclusion. Currently, improving the effectiveness of breast cancer treatment is mainly related to the optimal use of classic therapeutic strategies. New classes of substances have been approved for treatment or are in advanced stages of clinical development.
Keywords. neoadjuvant therapy (NCT), breast cancer (BC), triple negative breast cancer (TNBC)

Introduction

This work consists of a review of reports from the last 5 years regarding progress in breast cancer (BC) diagnostics and treatment.[1-46] In particular, progress in reference to neoadjuvant therapy (NCT). BC is a heterogeneous disease with morphological and molecular features that influence prognosis and response to treatment. NCT therapy is standard in patients with locally advanced BC and in inflammatory BC.

The goals of NCT implementation

- Down staging of large, unresectable tumors to sizes that enable surgical resection.
- Reduction in the size of the resected area.
- Ability to perform breast reconstruction or to plan reconstruction.
- Implementation of immediate treatment without convalescence after surgery which is necessary when the first form of therapy is a surgical operation.

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 12.10.2018 | Accepted: 29.10.2018
Publication date: June 2019

Ostańska E, Bartusik-Aebisher D, Aebisher D, Kaznowska E. *Neoadjuvant therapy in breast cancer – objectives and tasks*. Eur J Clin Exp Med. 2019;17(2):153–156. doi: 10.15584/ejcem.2019.2.8

– NCT destroys any micro-metastases which limits the risk of a tumor spreading.
– The ability to monitor response to treatment and individualization of further proceedings.

In the absence of responses to treatment, the inclusion of patients in clinical trials for new drugs is undertaken. The evaluation of the response to treatment with new drugs may serve as a marker (surrogate) of routine therapeutic progress.[9,11] Diagnostic material obtained by core biopsy, mammotomy biopsy (mammotome biopsy MB), stereotactic biopsy (stereotactic biopsy-SBB0), estrogen receptor (ER), progesterone receptor (pr), human epidermal growth factor receptor-2 (HER-2) and proliferation index (Ki67). Depending on the condition of these parameters, the patient receives hormonal trapping, cytostatics or immunotherapy. Currently, the strategy of therapeutic treatment is the assessment of predictive factors which aim to apply optimal treatment, and are prognostic, in order to predict the time frame of the disease and without therapy. The main prognostic factors in BC are: tumor size, number of lymph node metastases, histological grading and status of hormone receptors and HER-2 receptor, and Ki67 proliferative index. Other additional parameters include the presence of DNA ploidy, mutation of the p53 gene, cyclin-E, the presence of tumor cells in the peripheral blood and bone marrow, vascular invasion and perineural spaces. These parameters, including the presence of lymph node metastases, currently determine the type of NCT therapy in BC. The effectiveness of NCT therapy depends on the type of treatment implemented. Despite the implementation of therapy based on these parameters, in some cases resistance to treatment occurs. There is hope in research on predictive and prognostic factors based on disorders at the cellular level.[31,38,43,45] In patients with hormone-dependent cancer (ER +, PR +, HER-2) and without lymph node metastases, molecular profiles may be used (MammaPrint, Oncotype DX, Prosigna, Brest Cancer Index (BCI), Endo Oredict Clin, Pam 50, PEPI , uPA, PAI-1.[2,4,9,24,27,35,40,46,47] They elicit patients with low risk of relapse who do not need a follow-up chemotherapy and patients at risk of relapse. The prognostic and predictive value is associated with the three-dimensional tumor (Tumor volume-Tv), which correlates with the presence of metastases to the lymph nodes. Tv is a better indicator of the presence of metastases than T-assessment.[1] Functional Tumor Volume (FTV), measured using Magnetic Resonance Imaging (MRI), seems to be a strong predictor in the assessment of cancer recurrence after NCT. It can also be used to assess pCR as well as postoperative pathomorphological assessment. The use of MRI (Magnetic Resonance Imaging) can be used as a method of assessing the effectiveness of NCT treatment depending on the cancer subtype. Evaluation is the pattern of shrinkage of tumor mass. It can be concentric, nodular, or mixed all of which can be seen in MRI. The pattern and intensity of tumor reduction can serve as an indicator of early response after NCT. There is a correlation with the BC biological subtype.[3,35,37,44] Breast MRI and Molecular Breast Imaging (MBI) are imaging methods that allow for non-invasive assessment of BC construction, pathophysiology and biology. BC cells, in order to obtain energy, reprogram cell metabolism. These processes can become the target of therapy. It can become a source of biomarkers used in prognosing and monitoring treatment. Based on the use of these imaging methods, early response to the NCT used can be identified. This allows one to modify the treatment. Research is still ongoing.[3,11,13,29,32] There is a locally advanced BC relationship with type of vascularization, which can be assessed in MRI. Asymmetric crayfish (AIBV-Increase In Breast vascularity) is more aggressive but more susceptible to NCT than BC with symmetrical vascularity.

Persistent AIBV after NCT, even if the tumor decreases, is worse and requires more intensive NCT.[28] Recent research has uncovered new therapeutic strategies based on the evaluation of the androgen receptor (AR). In triple-negative carcinomas (TNBC), despite obtaining a pCR after NCT therapy, the presence of AR makes them prognosticate better than AR negative cancers.[31,38] Tumor Infiltrating Lymphocytes-TIL have a predictive and prognostic value in BC TNBC or HER2+. In studies, patients with higher levels of TIL had better therapeutic effects. The survival time of patients with NCT trastuzumab and derivatives also increased. It may also herald extensive research into BC immunotherapy.[11,14,22,36,41] The course of TNBC is aggressive compared to other cancers. There is also no correlation of tumor size with the presence of lymph node metastases. TNBC is considered a cancer belonging to the BRCAness group. It is characterized by profiles as in cancer with the BRCA-1,2 mutation.[30,36] Research is under way on predictive and predictive factors that may play a role in the treatment of PARP inhibitors (platinum derivatives). Mainly in the treatment of NCT TNCA BRCAness and BRC-1,2 mutations.[7,21,23,24,26] Recent reports speak about the expression of mRNA in BRCA-1,2 negative carcinomas. It can be a predictor of NCT with anthracyclines.[10,18] There are reports of changes in the primary-immunohistochemical profile. This applies to the ER, PR and HER-2 receptors. Therefore, it is recommended to evaluate receptors in a tumor that has undergone NCT and to evaluate receptors in lymph node metastases. This is to check the actual state of the receptors. The patients who had a PIK3CA mutation after NCT had less chance of survival than those that have lost the mutation.[39] Some reports indicate that there is a relationship between the high values of KI-67 in patients who are to receive NCT. These patients have TNBC and BC Her-2+ and receive

anthracyclines and taxanes. The higher the KI-67 value, the more likely the pCR is after using these chemotherapeutics. This also applies to hormone-dependent cancers with high kI-67. The inclusion of chemotherapy in these patients results in a higher percentage of pCR.[19,20]

Conclusion

Currently, improving the effectiveness of breast cancer treatment is mainly related to the optimal use of classic therapeutic strategies. New classes of substances have been approved for treatment or are in advanced stages of clinical development. It is very important to establish molecular predictors for these substances. It will help physicians to find the best therapeutic option

Acknowledgments

Dorota Bartusik-Aebisher acknowledges support from the National Center of Science NCN (New drug delivery systems-MRI study, Grant OPUS-13 number 2017/25/B/ST4/02481).

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.9

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 157–161

REVIEW PAPER

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The role of opportunistic *Corynebacterium* spp. in human infections

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ABSTRACT

Introduction. The non-diphtherial corynebacteria (diphtheroids, "coryneform" bacteria) have been increasingly recognized as causative agents of human infections.

Aim. To provide an overview of the role of non-diphtherial *Corynebacterium* species in human infections.

Material and methods. Analysis of the literature data found in the PubMed database.

Results. The role of diphtheroids - inherently low-virulent microorganisms considered members of the human microbiota – as potential pathogens has been linked to specific risk factors including immunosuppression, implantation of biomaterials and invasive medical procedures. Their pathogenic potential is primarily associated with frequent multidrug resistance, the ability to adhere to biotic and abiotic surfaces and/or to form biofilm as well as with internalization, intracellular survival and persistence within human cells. The most common infections include bacteremia, sepsis, endocarditis, meningitis, urinary tract infections, respiratory tract infections, wound and skin infections, and endophthalmitis. The leading species are *C. jeikeium*, *C. striatum, C. urealyticum, C. amycolatum,* and *C. pseudodiphtheriticum*.

Conclusion. Opportunistic corynebacteria can be responsible for a wide range of infections which can be expected to increase in frequency in the future due to an enlarging population of patients with predisposing risk factors but also due to the increasing problem of antibiotic resistance in this group of bacteria.

Keywords. coryneform, diphtheroids, opportunistic corynebacteria

Introduction

The non-diphtherial corynebacteria (also known as diphtheroids, or "coryneform" bacteria) have been increasingly recognized as causative agents of opportunistic and nosocomial infections within recent years.[1-7] The bacteria are considered members of the human microbiota.[5-7]

Aim

The aim of this review is to discuss the role of non-diphtherial corynebacteria as potential human pathogens.

Description of the subject literature

The review was prepared by the analysis of the literature data found in the PubMed data base using the following

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 3.01.2019 | Accepted: 12.02.2019
Publication date: June 2019

Olender A, Bogut A, Bańska A. *The role of opportunistic "Corynebacterium" spp. in human infections.* Eur J Clin Exp Med. 2019;17(2):157–161. doi: 10.15584/ejcem.2019.2.9

keywords: diphtheroids, coryneform, opportunistic corynebacteria, *Corynebacterium jeikeium*, *Corynebacterium striatum, Corynebacterium amycolatum Corynebacterium minutissimum, Corynebacterium urealyticum, Corynebacterium pseudodiphtheriticum*, antibiotic resistance. This literature analysis spans the years 1990 to 2019.

Literature analysis

The role of non-diphtherial corynebacteria - inherently low-virulent microorganisms - as potential pathogens has been predominantly linked to specific risk factors. The compromise of the host immune system is considered the leading predisposing factor. Immunosuppression is observed in many patient groups including patients with the bone marrow suppression, malignancies, in transplant recipients, those who are treated with corticosteroids and in heavily antibiotic-experienced patients. The development of opportunistic infections is also favored in patients suffering from debilitation, diabetes, extensive trauma, wounds, burns, and HIV infection. Implantation of vascular grafts, prosthetic heart valves, and joint endoprosthesis also increases the risk of infection development. Invasive medical procedures posing a risk of the introduction of the skin and/or mucosal microbiota into sterile body sites are considered an additional risk factor.[4,5,8,9]

In addition to host factors, microbial determinants of pathogenicity contribute to the increasing role of diphtheroids as medically relevant microorganisms. Although the pathogenesis of non-diphtheriae *Corynebacterium* infections remains poorly understood and little is known about their virulence factors, there are several aspects linked to their increasing pathogenic potential. The leading aspect of this potential is a frequent multidrug antibiotic resistance of coryneform bacteria. The ability to adhere to biotic and abiotic surfaces and/or to form biofilms in which bacteria are protected both against antimicrobial agents and the host immune responses are also considered an important strategy promoting the involvement of bacteria in both medical devices- and tissue-associated chronic infections.[2,3,5,7,9-15] Moreover, recently, the *in vitro* pattern of adherence, internalization, intracellular survival and persistence of *C. pseudodiphtheriticum* within human epithelial cells has been demonstrated.[16]

The most frequent infections caused by opportunistic *Corynebacterium* spp. include bacteremia, sepsis, endocarditis, meningitis, urinary tract infections, respiratory tract infections, wound, skin infections and endophthalmitis (Table 1). Based on the literature data, reporting cases of bacteremia in hospitalized patients, it can be concluded that the leading species involved in the etiology are *C. jeikeium*, *C. striatum,* and *C. amycolatum*.[17-19]

Treatment of bacteremia caused by *C. jeikeium* can be problematic due to its resistance to a variety of antibiotics including beta-lactams, macrolides, licosamides, tetracycylines, aminoglycosides. Nevertheless, bacterial strains belonging to this species tend to remain sensitive to vancomycin. Satisfactory therapeutic effects have also been observed following administration of daptomycin – a novel lipopeptide antibiotic, although daptomycin-resistant strains have already been identified.[20]

Bacteremia is also important in the development of infective endocarditis. Clinical manifestations of endocarditis caused by diphtheroids initially can be of low specificity and follow a chronic course. *C. jeikeium* is a species which deserves special attention when prosthetic valve endocarditis is concerned. In cases analyzed, the period between the onset of symptoms and the diagnosis of infectious endocarditis (defined as subacute) usually ranged between 1 to 3 months. In early endocarditis, in turn, the symptoms developed in more than half of the patients within 60 days after the prosthetic valve implantation; the mortality rate was 38%, whereas late endocarditis was associated with a mortality rate of 33%.[21]

C. striatum is another species involved in the etiology of prosthetic valve endocarditis. A long hospital stay has been identified as an important risk factor for the development of this type of infection. The association between placement of prosthetic valves and the isolation of *C. striatum* from the site of the infection was observed for 50% of analyzed patients. [22] Another important issue associated with endocarditis of this etiology, was the development of embolism in up to one third of patients.[1]

C. amycolatum can also be involved in the etiology of bacteremia and infective endocarditis. Due to a frequent multidrug resistance of this species, import information on the effective treatment is presented in the case report in which daptomycin was combined with rifampicin.[23]

Table 1. The most common sites of human infections caused by the opportunistic *Corynebacterium* species

Sites of infection	***Corynebacterium*** **species**
– bacteremia – endocarditis – infections with different locations	*C. jeikeium* *C. striatum* *C. amycolatum*
– urinary tract – infections with different locations	*C. urealyticum*
– respiratory tract – infections with different locations	*C. pseudodiphtheriticum*
– skin – infections with different locations	*C. minutissimum*

C. minutissimum is the frequent causative agent of life-threatening infections associated with bacteremia in neutropenic, HIV- infected patients, transplant recipients or in patients suffering from malignancies. Infections caused by this species can be invasive, and present as meningitis, glomerulonephritis, or cellulitis. In analyzed cases the portals of entry were most probably central venous catheters, peritoneal dialysis, hemodialysis. Most infected patients were effectively treated with vancomycin.[24,25]

C. coyleae, represents a diphtheroid species linked to bacteremia in immunocompromied patients; it is frequently isolated from the bloodstream of hospitalized patients. The predominant types of infections caused by this species include cellulitis, bacteriemia in adults and neonates, sepsis. *C. coyleae* strains can demonstrate MLS_B resistance phenotype (macrolide-lincosamides-group B streptogramins), conferred by the *ermX* gene.[26]

It should be noted that diphtheroid species identification is nowadays facilitated by molecular methods. Their implementation in the microbiological diagnostics allowed to identify new *Corynebacterium* species, mainly isolated from the blood and exemplified by *C. tuscaniae, C. ureicelerivorans, C. timonensez, C. falsenii,* and *C. musteale*.[27-31]

Opportunistic *Corynebacterium* ssp. can also be involved in the etiology of urinary tract infections (UTIs). The predominant species is a lipophilic *C. urealyticum* associated with UTIs in immunocompromised patients, patients who underwent urological procedures or those with previous episodes of UTIs. Infections caused by *C. urealyticum* are usually diagnosed in the hospital setting and it is assumed that they have an association with the previous colonization of the skin. Important hallmarks facilitating the recognition of this etiologic agent of UTI include the presence of the magnesium-ammonium phosphate (struvite) crystals in the urine which contribute to the formation of renal stones and alkaline urine pH resulting from the profuse production of bacterial urease.[32-35]

Large amounts of the produced struvite crystals can be deposited on the wall of the bladder which can subsequently lead to the development of encrusting cystitis which frequently follows a chronic course, and is associated with interference in urine flow, dysuria, suprapubic and flank pain, and hematuria. Fever develops only in 25-50% of infected patients. The diagnosis is based on the examination of area and thickness of calcifications on the surface of the bladder mucosa. In reported cases, infected patients had been previously subjected to the diagnostic or therapeutic urinary procedures which facilitated transmission of *C. urealyticum* for the skin. The encrusting cystitis or pyelonephritis were recognized between 5 days and 3 years after the aforementioned urological procedure.[31,35]

C. urealyticum can demonstrate different antibiotic resistance profiles including multidrug resistance. Nevertheless, it is commonly sensitive to vancomycin and teicoplanin which is effectively used in the treatment of infections caused by this species.[34,35]

Respiratory tract infections (RTIs) are most commonly caused by *C. pseudodiphtheriticum* – a member of the normal microbiota of the human nasopharynx. It tends to cause RTIs in immunocompromised patients and in cystic fibrosis patients. It can also cause nosocomial infections in patients with tracheal intubation. In the reported cases of infections caused by *C. pseudodiphtheriticum* particular attention has been drawn to the predisposing factors including congestive heart failure, chronic renal insufficiency, diabetes, malignancy, obstructive pneumonia, previous infection with *Chlamydia pneumoniae*. In one thirds of infected patients the diphtheroid infection was accompanied by the rise in the body temperature > 37°C and an acute course. *C. pseudodiphtheriticum* has been isolated from the sputum but not from the bloodstream.[37,38]

The second most frequent diphtheroid species involved in the etiology of RTIs, especially in the hospitalized patients, undergoing long courses of antibiotic therapy, is *C. striatum*. This species includes 14 genotypes. Strains representing types A, D, and E have been predominantly associated with hospital-acquired infections. Subtypes A1, A2, D2, and E tend to be resistant to erythromycin, tetracycylines, rifampicin, ciprofloxacin, and they present a varied degree of resistance against beta-lactams and aminoglycosides. Similarly to other diphtheroid species, in turn, *C. striatum* remains sensitive to vancomycin. *C. striatum* is considered an important multidrug resistant pathogen which is transmitted between hospitalized patients and medical personnel. Its involvement in human infections is also frequently associated with catheterization, intubation and immunosuppression.[39,40]

It has been estimated that *Corynebacterium* species inhabiting the human skin account for more than half of microorganisms constituting the skin microbiota devoid of significant pathogenic potential. Skin infections can be caused by *C. minutissimum* – the causative agent of erythrasma. This species leads to development of scaly lesions in the axilla which can be misdiagnosed as a fungal infection. Nevertheless, this diphtheroid can also cause mixed infections along with dermatophytes and *Candida albicans*. Moreover, *C. minutissimum* can be transferred from the erythrasma foci and, particularly in immunocompromised individuals, it can lead to development of bacteremia, meningitis, endocarditis, cellulitis, or pyelonephritis.[24,41,42]

Pitted keratolysis is another example of the skin infections caused by diphtheroids. This type of infection does not involve deeper layers of the skin which was evidenced by the observation of clinical samples under the electron microscope. The presence of bacteria was

detected within keratinocytes, and granular changes of keratohyalin were associated with the proteolytic activity of bacteria. Other corynebacteria species involved in the development of skin lesions include *C. striatum* (extensive, erythema lesions with irregular edges), *C. pseudodiphtheriticum* (ulcers). The non-diphtherial corynebacteria can additionally be involved in the development of chronic wound infections.[43,44]

Implant and prosthetic joint infections are indicative of adherence capabilities of *Corynebacterium* spp. It has been reported that *C. massiliense* isolated from the synovial fluid was associated with the prothetic joint infection, whereas *C. xerosis* was involved in the etiology of the cardioverter-defibrillator implant and led to the development of bacteremia.[29,45,46]

Opportunistic corynebacteria also constitute a significant problem in ophthalmology. Literature data present cases of endophtalmitis associated with this group of bacteria.[47-50] For example, Ferrer et al. have discussed a case of postoperative *C. macginleyi* endophtalmitis. This microorganism was identified with the use of the PCR assay and the analysis of the 16rRNA gene sequence with the lack of growth in the conventional culture.[47] Quin et al., in turn, reported a case of posttrabeculectomy bleb-related infection associated with *Corynebaterium macginleyi*.[51]

Conclusions

Based on the literature, the non-diphtheria corynebacteria can be responsible for a wide range of human infections which can be expected to increase in frequency in the future due to an enlarging population of patients with predisposing risk factors but also due to the increasing problem of antibiotic resistance in this group of bacteria.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.10

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 162–168

REVIEW PAPER

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Labial salivary gland biopsy in the diagnosis of Sjögren's syndrome

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ABSTRACT

Introduction. Labial salivary gland biopsy is used for diagnosis of Sjögren's syndrome (SS) and lymphoma accompanying SS.

Aim. The aim of this study was to present the main techniques used for taking labial salivary gland biopsies in the diagnosis of SS with respect to their advantages, histologic criteria, validation, complications, and their usefulness for diagnostic procedures, monitoring disease progression, and treatment evaluation.

Material and methods. This study is based on analysis of literature.

Results. The microscopic confirmation of SS is based on the presence of focal lymphocytic sialadenitis (FLS) with a focus score ≥1 per 4 mm^2 of glandular tissue. A lymphocytic focus is defined as a dense aggregate of 50 or more lymphocytes adjacent to normal-appearing mucous acini in salivary gland lobules that lacked ductal dilatation. Other histopathological features of SS are lymphoepithelial lesions and a relative decrease of <70% IgA + plasma cells. Labial salivary gland biopsy is characterized by high specificity, a positive predictive value, and an average sensitivity of 79% in SS.

Conclusion. It can be also valuable in diagnosing B-cell mucosa-associated lymphoid tissue (MALT) lymphomas but it is not recommended for the monitoring of SS progression and the effectiveness of the treatment. Persistent lower lip hypoesthesia is the most severe complication of labial salivary gland biopsy.

Keywords. biopsy, labial glands, salivary glands, Sjögren's syndrome

Introduction

Labial minor salivary gland biopsy (LSGB) is used for the diagnosis of systemic disorders, such as amyloidosis, sarcoidosis, Sjögren's syndrome (SS), lymphoma accompanying SS, and other connective tissue disorders, and also to confirm neonatal haemochromatosis.[1] The final classification criteria of SS, which was approved by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) in 2016, is based on the weighted sum of 5 items: anti-SSA/Ro antibody positivity and focal lymphocytic sialadenitis (FLS) with a focus score of 1 foci/4 mm^2, each scoring 3; an abnormal ocular staining score of 5 (or a van Bijsterveld score of 4), a Schirmer's test result of 5 mm/5 minutes, and an unstimulated salivary flow rate of 0.1 ml/minute, each scoring 1. Individuals with signs and/or symptoms suggestive of SS who have a total score of 4 for the above items meet the criteria for primary SS.[2,3] Although LSGB is considered a minor procedure, its results may have a significant impact on the diagnosis

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 12.01.2019 | Accepted: 15.02.2019
Publication date: June 2019

Błochowiak K, Sokalski J. *Labial salivary gland biopsy in the diagnosis of Sjögren's syndrome*. Eur J Clin Exp Med. 2019;17(2):162–168. doi: 10.15584/ejcem.2019.2.10

of SS, and the lack of uniformity in methodology and potential adverse effects have hindered its application. There is no standardized technique that yields adequate tissue for analysis and minimizes adverse effects.

Aim

The aim of this study was to summarize the main techniques used for taking labial salivary gland biopsies in the diagnostic workup of SS with respect to their advantages, histologic criteria, validation, complications, and their usefulness for diagnostic procedures, monitoring disease progression, and treatment evaluation.

Histologic criteria for diagnosis of SS on labial salivary gland biopsies

SS is an autoimmune disease characterized by chronic T-and B-cell infiltration of the salivary glands or lachrymal glands, leading to exocrine gland dysfunction with symptoms and signs of dry mouth and keratoconjunctivitis sicca.[4-11] Patients may present with variable combinations of systemic extra-glandular manifestations such as peripheral neuropathy, arthralgia and lung disease. SS is often difficult to diagnose, as the clinical and laboratory manifestations vary widely. None of the laboratory markers are both sensitive and specific. However, several sets of classification criteria have been developed over the last few decades. All these sets combine clinical findings, serological tests, and a histological evaluation of salivary gland involvement. In all previous classifications, both objective and subjective tests were included in diagnosis of SS.[4-14] The current classification is based only on an objective clinical, serological and histopathological test. LSGB is an objective test of SS and plays a significant role in the diagnostic process. In fact, the presence of either anti-SSA/SSB seropositivity or a positive lip biopsy is a requirement for an individual to be classified as having SS. The microscopic confirmation of SS is based on the presence of focal lymphocytic sialadenitis (FLS) with a focus score ≥1 per 4 mm^2 of glandular tissue.

According to the revised American-European Consensus Group's (AECG) classification criteria and the ACR classification criteria for SS, a labial salivary gland biopsy is considered positive if minor salivary glands demonstrate FLS, with a focus score of 1 or more, as evaluated by an expert histopathologist. A lymphocytic focus is defined as a dense aggregate of 50 or more lymphocytes adjacent to normal-appearing mucous acini in salivary gland lobules that lacked ductal dilatation.[2,3,15,16] FLS is applied to specimens that show the presence of 1 or more foci of lymphocytes located in periductal and perivascular locations. The foci can contain plasma cells, but these must be a minority constituent of the inflammatory infiltrate. The focus score can be calculated for those specimens showing the histopathologic appearance of FLS. The number of lymphocytic foci is then determined for all the gland lobules in a single tissue section. The focus score is then calculated as the number of foci per square millimeter of glandular tissue multiplied by four, which then yields foci/4mm^2. A focus score of 1 equates to 1 focus/4 mm^2. To determine the focus, a calibrated eyepiece grid or image analysis software with a closed polygon tool is used. FLS has to be distinguished from nonspecific chronic sialadenitis. The symptoms of non-specific sialadenitis are mild to moderate acinar atrophy, interstitial fibrosis, and ductal dilatation, with lymphocytes and macrophages often scattered in the parenchyma, but not forming dense aggregates of 50 or more lymphocytes immediately adjacent to normal-appearing acini.[15] In addition to the focus score (FS), two scoring systems for salivary glands are in use for the diagnosis and classification of SS. These systems are based on the presence of foci. Grading according to Tarpley's system involves destruction of acinar tissue and fibrosis.[17] Grading according to the Chisholm and Mason system is based on the presence of infiltrates from slight to one or more foci.[18] There are also other histopathological features in the labial glands that are associated with SS and therefore might be indicative of this disease. Lymphoepithelial lesions (LELs) are striated ducts, which are infiltrated by lymphocytes with concurrent hyperplasia of the epithelial cells. They are found both in parotid and labial glands, and are more representative of parotid glands than labial glands. Besides LELs, the salivary gland of SS patients also presents a relative decrease in IgA^+ plasma cells. Several studies showed that a relative decrease of <70% IgA^+ plasma cells was more sensitive and more disease specific than the FS. Both features can help assess the salivary gland biopsies for the diagnosis of SS, especially when the FS in the biopsy is <1.[19] The tissue specimens should be immediately placed in a wide-mouthed container, coded, and fixed in a generous amount of 10% formalin buffered saline for 24h. There is no standardization of labial salivary gland biopsies in SS, but Fox noticed several points of importance in LSGB.[19] The first issue refers to a sufficient amount of glandular tissue. A reasonable compromise is four glands, although a minimum sized evaluable surface area (8mm^2) may be achieved with 2-3 glands. The largest possible area to be sampled would give the best results, but a larger operative field increases the surgical risk. On the other hand, some glands may be atrophic or damaged, and the volume of the material obtained through the biopsy should be sufficient to overcome this artefact and achieve a valid result. It is more recommended to evaluate multiple different lobules than to concentrate on a single abnormal lobule, which may not be typical of the entire gland. In routine management, H&E staining is used in order to determine these structures. For clinical trials, addi-

tional staining with CD21 as well as CD20 and CD3 is required. CD21 is a marker of follicular dendritic cells. Germinal centers should be reported and pathologists are advised to use caution in order to avoid overestimating germinal centers by relying solely on CD21.[20,21] Furthermore, the distribution of the inflammatory cells in the gland may be uneven. Considering this uneven distribution, a single tissue section may result in underdiagnosis. While increasing the number of sections has the potential to reduce this problem, the optimal number of sections has yet to be determined. Some research suggests taking labial salivary glands at different depths from the same incision. FS can change significantly at different tissue depths within the minor salivary glands. Multiple sections for LSGB increase the diagnostic value and are more representative than a single section.[22]

Indications for LSGB and its usefulness in SS

LSGB is characterized by quite high specificity, a positive predictive value, and an average sensitivity of 79% in SS.[23] In other studies, the sensitivity and specificity are reported at 86.7% and 97.4%, respectively.[24] The sensitivity and specificity of labial salivary gland biopsies vary in the literature. Data from different studies are often difficult to compare, because different sets of criteria for diagnosing SS have been used and the outcome of the labial biopsy is a strong determinant for the final diagnosis. In the normal population, labial biopsy resulted in 6% to 9% false-positive diagnoses; 18% to 40% of the patients with a clinical diagnosis of SS have a negative labial biopsy, resulting in a sensitivity of 60% to 82% and a specificity of 91% to 94%.[25] In some cases, a positive histologic confirmation in LSGB does not correspond with serologic positivity for SSA or SSB. Thus, clinicians avoid performing LSGB in most patients with positive SSA/SSB serology.[26,27] On the other hand, clinical presentation of sicca symptoms and positive serology reliably predicted the results of a lip biopsy.[26] Taking both symptoms and serology into consideration is more likely to yield an accurate clinical picture than either one alone. Several studies have questioned the utility based on the invasiveness of the procedure and the high rate of pathologic misinterpretation.[25] Moreover, patients with a typical presentation of SS do not derive any additional benefit from a lip biopsy. A positive serologic result and a positive ocular test make the taking of a LSGB redundant and only in case of a negative serologic outcome or a negative result in the ocular test is a LSGB is indicated.[28] These divergent results are reported mainly in the initial stages of SS or in patients with low focus score.[26] Lack of adequate tissue can also lead to misdiagnosis or lack of diagnosis. Moreover, a possible cause of these divergent results between clinical and serological symptoms and LSGB could be the fact of taking immunosuppressive medications and steroids. There is a stronger correlation between the lip biopsy and clinical presentation of sicca with positive serology, suggesting that corticosteroids may have a tendency to confound biopsy results. The use of high-dose corticosteroids can not only relieve a patient's symptoms of SS, but also decrease the lymphocytic infiltrate of a second minor salivary gland biopsy. To avoid the confusion of false negatives, clinicians should be wary of performing a lip biopsy in patients on immunosuppression with clear criterion for SS.[25] High specificity and sensitivity make LSGB particularly useful for patients with inconclusive clinical findings, incipient forms of the syndrome, SS with negative anti-Ro/la serology, and extra-glandular involvement.[25] Moreover, LSGB can be valuable in diagnosing B-cell mucosa-associated lymphoid tissue (MALT) lymphomas, which very rarely accompany SS. 4% to 7% of patients with SS develop malignant B cell lymphoma, 48% to 75% of which are of the MALT type. These B-cell lymphomas are more frequently located in the parotid glands than in labial glands.[29] Furthermore, LSGB may be a very useful in diagnosis of SS in children. Histopathological evidence of typical FLS is also considered by some pediatric rheumatologists to be the gold standard in the diagnosis of childhood SS.[30] Unfortunately, LGB is not recommended for monitoring disease progression and treatment evaluation.

Anatomical implications and complications

Minor salivary glands are widely distributed in the labial, buccal, and palatal mucosa of the oral cavity. Microscopic findings involving lymphocytic infiltration surrounding the excretory ducts in combination with the destruction of acinar tissue are representative for all minor salivary glands and are pathognomonic changes for SS. Lip salivary glands are largely used for assisting the diagnosis of SS, because they are easily accessible and lie above the muscle layer. They are separated from the oral mucous membrane by a thin layer of fibrous connective tissue. Orientation and identification of glandular tissue is easiest. The risk of excessive postoperative bleeding is decreased because the arterial supply to the lip lies deep.

One of the most severe complications of LSGB is sensitive nerve injury. This localized sensory alternation can be described as an anesthesia, a reduced or partial loss of sensation, a transitory numbness or a hypoesthesia. These sensations can last for a few months or can be permanent. Persistent lip numbness occurs in up to 6% of biopsies performed in the lower lip.[3] The branches of the mental nerve in the lower lip are closely associated with the salivary glands and this anatomical relationship increases the risk of postoperative sensory sensations. Additionally, the branch of the mental nerve usually divides into 2 sub-branches: a horizontal and a vertical, which have an ascending course toward the vermillion

border and are in close relation to the labial salivary glands. Incisional biopsies shorter than 2 cm performed with a scalpel have reported complications ranging from 0% to 9.3%, whereas those using larger incisions (2–3 cm) have described complications in the range of 3.7–31%. Transient disorders of lip sensitivity are found to occur in up to 11.7% of procedures. Persistent lower lip hypoesthesia is reported in about 3.4–4% of cases.[23] Larger incisional biopsies and punch biopsies are associated with a higher risk of both transient and persistent lower lip numbness. Other possible complications of LSGB are less severe, usually transient or temporary, and are associated with localized postoperative inflammation or improper healing. The symptoms of postoperative inflammations are local pain and swelling. Blood vessel injuries result in external hematoma. The possible delayed complications are the formation of granulomas, internal scarring and cheloid formation. Some patients can report burning or tingling sensations, and functional deficits during the immediate postbiopsy period such as eating, sleeping or speech difficulties.[15]

Surgical technique and approaches

Labial gland biopsy can be a excisional or incisional technique. The most recommended site is normal–appearing mucosa of the lower lip. A wide range of surgical approaches have been described for harvesting a few accessory glands from the lower lip using different instruments such as a scalpel, a punch or cup forceps. The use of a forceps with a fenestrated active end to stabilize the lip has also been suggested.[3] The excisional biopsy is carried out by excising an ellipse of oral mucous membrane down to the muscle layer. Ideally, 6 to 8 minor glands must be harvested and sent for histopathologic examination. The wound should be closed with 4-0 silk sutures, which are removed after 4 to 5 days. The modification of this method is the technique with a mucosal excision of 3.0 × 0.75 cm. Another recommended technique is a 1.0 to 1.5 cm wedge-shaped excision of the mucosa between the midline and commissure. The incisional biopsy is described as a 1.5–2.0 cm linear incision of mucosa, parallel to the vermillion border and lateral to the midline. Gorson and Ropper reported a 1-cm vertical incision just behind the wet line through the mucosa and submucosa.[31] It is usually that case that the lateral lip compartments are advocated for biopsy, because of the glandular-free zone in the center of the lower lip. Berquin et al., described an oblique incision, starting 1.5 cm from the midline and proceeding latero-inferiorly to avoid the central glandular-free zone.[32] According to Saruhanoğlu et al., the vertical incision technique is associated with less pain, less swelling, less scar formation and less difficulty in eating when compared with the horizontal incision technique.[33] There is insufficient evidence to support the superiority of one technique over the others and the shape and the size of the incision can be considered a matter of preference.[34] The incision shape includes elliptical, circular, linear, horizontal, vertical, and wedge shapes and the incision length varies from a few millimeters to 2 cm.

Another recommended modification using loupe operation glasses to precisely excise the salivary glands without disturbing the direct underlying sensible nerves. The alternative technique to scalpel biopsy is the minor salivary gland punch biopsy. This biopsy can be performed by a single operator, and it is less expensive than classical scalpel biopsy. This technique consists of obtaining the biopsy from the buccal side of the lower lip, which is stabilized by the patient him/herself using a 4-5 mm punch, which permits the retrieval of a cylinder of tissue up to 8 mm in length.[5] The punch biopsy is suggested because of the absence of risk to the patient and because of its simplicity. However, according to Varela-Centelles et al., punch biopsies did not provide enough material for diagnosis of Sjögren's syndrome. Moreover, the findings of this study strongly discouraged the punch technique for minor salivary gland lip biopsy and provided information on the superiority of the linear incisional biopsy in terms of neural damage.[23]

Based on our own clinical experience, I suggest a 1.0 to 1.5 cm linear, horizontal incision of mucosa parallel to the vermillion border and lateral to the midline with the tip of a 15 scalpel. The lower lip should be retracted and everted under tension to expose the inner surface and allow visualization of the minor salivary glands just to the depth of the mucosa. Local anesthesia injected submucosally with 0.5 to 1.0 ml of 1% lidocaine with 1:200000 epinephrine is sufficient. The anesthesia hydro-dissects and lifts the mucosa away from the salivary glands, provides delivery of local anesthetic directly to sensory nerve fibers and temporarily displaces small vessels deep in the glands to promote hemostasis and visualization during the dissection. In this technique both margins of incision should be gently crafted to access the submucosal layer. This stage of procedure can be performed using blunt-tipped iris scissors by spreading in a plane perpendicular to the mucosal incision and parallel to the direction of the sensory nerve fibers. In my opinion this technique is fast, simple and leaves a small scar. The linear incision secures a good adherence of wound margins and proper and fast healing. Unfortunately, this method is not effective in small amounts of salivary glands. It is difficult to find the sufficient amount of labial glands. Moreover, it may be difficult to harvest a sufficient number of labial salivary glands in atrophic mucosa of patients with long-standing SS. Furthermore, the recommended method is a 1 cm lenticular incision of mucosa, lateral to the midline, and removal of the mucosa to uncover the submucosal layer and obtain a few adjacent salivary glands.

This technique ensures good visibility into the operating field to avoid blood vessels and nerve injures. This incision provides adequate glandular tissue for diagnosis. The wound should be closed by a few non-resorbable, single, interrupted stitches. One very important issue is to harvest only labial salivary glands without muscular or other tissues. It is the most valuable specimen for histopathological examination, because it only includes glandular tissue. Additionally, this technique decreases the risk of nerve damage and postoperative pain and assures successful healing. Sensory nerve fibers are almost always visible just below the plane of dissection and care should be taken to identify and preserve them. The next very important issue is not to puncture the labial glands in order to reduce the risk of mucocele formation. It is even better to remove all visible labial salivary glands from the operating field before suturing in order not to damage the glands or their ducts. Patients should also avoid taking steroids before the biopsy. The factors potentially contributing to a false-negative rate include the use of oral steroids that may result in immunosuppression and confound histopathologic results.[15]

Alternative salivary gland biopsies in SS

The selection of the best surgical approach in terms of related morbidity is hampered by the absence of comparative studies and the proliferation of descriptive papers that do not state negative outcomes associated with the technique used. Moreover, reports describing the percentage of surgical complications have limited validity due to the lack of standardizations when defining and categorizing the complications in accordance with their severity.

The main alternative types of salivary gland biopsies in SS are parotid gland biopsy and sublingual gland biopsy. Parotid gland biopsy allows the clinician to monitor the disease progression and to assess the effect of an intervention treatment in SS. Parotid tissue can be harvested easily, repeated biopsies from the same parotid gland are possible, and the histopathologic results can be compared with other diagnostic results derived from the same gland, such as secretory function, sialographic appearance and ultrasonography. Furthermore, parotid biopsy is better in the identification of lymphomas.[25] The main possible complications are facial nerve damage, Frey's syndrome and development of sialoceles and salivary fistulae. A temporary change in sensation in the skin area of the incision is also a well-documented complication after parotid biopsy. Some patients might also develop preauricular hypothesia, although this is usually temporary. Furthermore, in SS, the salivary gland tissue is replaced by fatty tissue, and the risk of harvesting fatty tissue is thereby increased if done by inexperienced physicians.[35,36] Parotid biopsy is particularly recommended in pediatric patients in whom SS is suspected and who have a negative minor salivary gland biopsy result. Incisional biopsy of the parotid gland overcomes most of the disadvantages of labial biopsy. When evaluating the parotid and labial biopsy, sensitivity and specificity are comparable, estimated to be 78% and 86%, respectively.[25,37] Comparative studies suggest that both procedures – sublingual and parotid biopsy – retain a diagnostic potential comparable to that of lip biopsy and may be associated with lower postoperative morbidity. A comparison of sublingual gland biopsy with labial gland biopsy is better than that of labial gland biopsy, whereas the specificity of the latter is greater than that of the former. Sublingual gland biopsy is a relatively safe procedure, although the postoperative complications of sublingual salivary gland biopsy include ligaturing the Wharton duct, resulting from the placement of sutures, bleeding and swelling in the floor of the mouth. Damage to the lingual nerve related to this biopsy technique has never been reported in the literature. No specialized histopathologic criteria have been established for the diagnosis of SS after a sublingual gland biopsy, and researchers merely used the criteria for labial gland biopsies.[25,38-40]

Conclusions

Labial salivary gland biopsy is an integral part of diagnosis of Sjögren's syndrome, but it has a limited value for monitoring of the disease progression and for an assessment of effectiveness of the treatment. The standardization of the surgical technique and the histopathological examination can increase the diagnostic value of the biopsy.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.11

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 169–174

REVIEW PAPER

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What is the unique nature of the Huntington's Disease?

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ABSTRACT

Introduction. Huntington's disease is a rare neurodegenerative disease, inherited in an autosomal dominant manner. Every child in the family whose parent is a carrier of the mutant gene has a 50% risk of inheriting the disease. Genetic tests unambiguously confirm whether a person at risk is ill or not. Symptoms include movement, neuropsychiatric and cognitive disorders. Currently, the disease is incurable and there are no effective methods for its treatment.

Aim. The aim is to present information about Huntington's disease, its inheritance, symptoms and pathologies, as well as to draw attention to its unique impact on patients and their families.

Material and methods. A literature review of the following databases has been conducted: PubMed, Science Direct, EBSCO, Springer Link.

Results. Huntington's disease, due to the autosomal dominant inheritance, disturbs the whole family system. Over several generations, a family can struggle with the problems of taking care of several patients at the same time, providing children with information about the risk of falling ill, making decisions about genetic testing, and starting a family or having children.

Conclusion. Huntington's disease is a challenge for healthcare professionals who are not always prepared to solve unique, multi-generational problems in families with Huntington's disease.

Keywords. CAG repeat, chorea, family system, genetic disease, HD gene

Introduction

Huntington disease (HD) is a rare, progressive neurodegenerative disease that belongs to a unique group of autosomal-dominant disorders. This disorder belong to trinucleotide repeat disorders and is caused by CAG trinucleotide repeats in the 5´ coding region of the IT15 (Interesting Transcript15) gene located on locus 4p16.3.[1,2]

The Huntington's gene has a unique feature of repeating a trinucleotide DNA (triplet) with a repeat length of 6 to 35 in the normal population.[3] The number of repetitions in the range of 27–35 does not cause the symptoms of the disease to develop, but due to the phenomenon of anticipation, it is associated with an increased risk of mutation in the next generations. The phenomenon of anticipation lies in the fact that the higher the number of repetitions, the earlier the age of illness and its more severe course.[4,5] It mainly concerns inheritance from father, which is the result of high in-

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 9.05.2019 | Accepted: 13.06.2019
Publication date: June 2019

Bartoszek A, Szalast K. *What is the unique nature of the Huntington's Disease?*. Eur J Clin Exp Med. 2019;17(2):169–174. doi: 10.15584/ejcem.2019.2.11

stability of CAG sequences in spermatogenesis. Genetic anticipation is the reason for the emergence of a juvenile form of HD in families. Every child in a family whose parent suffer from HD has a 50% risk of inheriting the gene. A range of 36-39 repetitions of a CAG triplet can cause the disease, but the gene penetration is incomplete.

For people with 40 and more repetitions, the penetration is 100%, which means that every such person will fall ill if he lives to a certain age.[6] After full-blown HD develops, patients live about 15-20 years, slightly longer than in other neurodegenerative diseases. In the family, it often occurs in several people at the same time, for example in the case of a spouse, children, grandchildren, and several generations even up to 30 years.

Aim

The aim of this paper is to present information about Huntington's disease, its inheritance, symptoms and pathologies, as well as to draw attention to its unique impact on patients and their families.

Description of HD from 1872

The name of the disease comes from the name of the doctor George Huntington, who in 1872 published an article named "On Chorea" in the Medical and Surgical Reporter. He gave a detailed description of the progressive and hereditary chorea, inexorably progressive, and always fatal: "When either or both the parents have shown manifestations of the disease, and more especially when these manifestations have been of a serious nature, one or more of the offspring almost invariably suffer from the disease, if they live to adult age. But if by any chance these children go through life without it, the thread is broken and the grandchildren and great-grandchildren of the original shakers may rest assured that they are free from the disease. This you will perceive differs from the general laws of so-called hereditary diseases, as for instance in phthisis, or syphilis, when one generation may enjoy entire immunity from their dread ravages, and yet in another you find them cropping out in all their hideousness. Unstable and whimsical as the disease may be in other respects, in this it is firm, it never skips a generation to again manifest itself in another; once having yielded its claims, it never regains them. In all the families, or nearly all in which the choreic taint exists, the nervous temperament greatly preponderates, and in my grandfather's and father's experience, which conjointly cover a period of 78 years, nervous excitement in a marked degree almost invariably attends upon every disease these people may suffer from, although they may not when in health be over nervous".[8] In several decades, the chorea described by Huntington was widely recognized as a unique disorder. The name was changed to Huntington's disease because it became obvious that the disease is not only associated with the occurrence of movement disorders.

HD gene

The identification of the HD gene was based mainly on the analysis of a large Venezuelan HD kindred with an extremely high frequency of the disease, due to the high frequency of inbreeding. The HD gene was mapped to the tip of the short arm of chromosome 4 in 1983 using standard linkage analysis. However, scientists needed another 10 years to isolate it and to identify the underlying mutation that causes HD. But it was not until 1993 when the HD gene was finally identified by The Huntington's Disease Collaborative Research Group, including 58 scientists from six independent research groups and introduced for diagnostic and preclinical tests.[9] Using the haplotype analysis of coupling disequilibrium in HD families of different ethnic groups, they identified a small segment of 4p16.3 as the probable location of the mutation. The new gene, IT-15, isolated using cloned trapped exons from the target area, was shown to contain a polymorphic trinucleotide CAG repeat within the coding region of the gene that was expanded and unstable on one of the chromosomes of all 75 HD families examined. The gene locus was found to span 180 kb, consisting of 67 exons, and encoding a protein (huntingtin) of ~350 kDa.[10] One year later the international clinical and genetic community has created the guidelines for the appropriate molecular genetic testing of HD.[11] HD is unique due to its single gene autosomal dominant nature and can be accurately identified in patients prior to the onset of any symptoms, while still considered 'premanifest'.[12]

Genetic tests and its consequences

People at risk, over the age of 18, may undergo a presymptomatic study to find out if they actually carry a mutated gene. This is a choice that selects few people at risk (5% to 10%) and requires a multi-day consultation with genetic counselors, neurologists, and often a psychiatrist or psychologist.[3]

Due to the long-term and difficult course of the disease, confirmation of clinical diagnosis of HD has unique consequences for the patient and other family members. A patient's reaction to the disease may be unpredictable.[13] A negative diagnosis sometimes helps to explain worrying symptoms and therefore is a relief for the patient and his family. However, HD diagnosis can cause or worsen an existing depression - the risk of suicide increases just before and after the beginning of the disease. Predictive testing is very important for people with a family history of HD before significant life-changing events such as marriage or pregnancy occurs.[14] If the diagnosis is confirmed, other people in the family (patient's partner, children, grandchildren,

siblings) will need information and emotional support, especially if there is no previously known family history of HD.[15]

Symptoms and forms of HD

Pathological findings in HD include mostly progressive degeneration of basal ganglia but also of other brain regions, such as the substantia nigra, cerebral cortex, hippocampus, lateral tuberal nuclei of the hypothalamus and parts of the thalamus.[16]

Clinical diagnosis of the disease is based on the observation of involuntary movements and insidious beginning of mood disturbance in individuals with family history of the disease. The symptoms include motor, neuropsychiatric and cognitive abnormalities that aggravate progressively.[17]

There are 3 HD forms differing in the onset, clinical picture and course of the disease. Classic form consists approximately 80% of patients and Juvenile (The Westphal variant of HD) affect people before 20 years of age. Late onset HD (10% of patients) begin after 60 years of age and the diagnosis of the disease is often never made. In the family history there is no information about the disease, which due to the variability of the clinical picture has not been diagnosed as HD, but as Alzheimer's, Parkinson's or other neurological disease, especially if it is the first situation in the family.[18] The de novo mutation accounts about 10% of cases.[19]

The most common age of onset (classic form) is the fourth and fifth decade, when life stabilization is achieved, when HD patients have already made the majority of professional, financial and family decisions related to, among others, the number of children. This HD form is characterized by the presence of a triad of symptoms: involuntary movements, behavioral and mood disorders, and cognitive impairment. These symptoms occur in patients of varying intensity and various compilations. The spectrum of motor symptoms in HD is broad and includes predominantly chorea involuntary movements, saccadic eye movement disorders, postural and balance disorders, dystonic and myoclonus movements, dysarthria, dysphagia, and Parkinson syndrome in the late stage of the disease.[20,21] These changes result from damage to the movement loop responsible for the regulation of muscle tone and the function of skeletal muscles and reflexes. Uncontrolled body movements cause HD patients to be perceived as people under the influence of alcohol and because of motor disorders; they may push or hit other person accidentally. Other, less known, but common and frequently occurring symptoms of HD are: uncontrolled weight loss, sleep disorders, circadian rhythm dysfunction, and dysfunction of the autonomic nervous system.[22] Movement disorders make it impossible to perform daily activities such as washing, dressing, using the toilet, cooking and eating meals. Dysarthria and dysphagia intensify during the course of the disease, which can lead to choking and aspiration pneumonia. All patients develop hypokinesia and muscle stiffness that lead to bradykinesia and severe akinesia.[23] The most common causes of death are pneumonia, injuries resulting from falls or suicides, which occur much more often than in the general population.[24]

Neuropsychiatric disorders are dominated by depression (about 40% of patients), dysphoria, psychomotor agitation, irritability, apathy, anxiety, suppression of behavior and euphoria.[25] Depression syndrome in people at the early stage of the disease may be the first symptom of the pathological process, but it can also react to stress resulting from awareness of the genetic burden of the disease. In HD patients, there was also a family predisposition for the occurrence of psychotic symptoms. In families where at least one person has the disease manifested by psychotic symptoms, the risk of developing psychosis in other members burdened with its disease is about four times higher than in families burdened with HD without symptoms of psychosis.[26] The presence of psychotic disorders may suggest schizophrenia. In 40-50% of patients, over a dozen years before the onset of the first symptoms of HD, personality changes are observed.[27] Irritability associated with HD, which is signaled by members of the immediate family, can be severe and result in outbursts of anger and aggression.[28] Over half of the patients develop obsessive-compulsive disorder during the disease course, and perseveration behavior (75% of patients). [29,30]

Symptomatology of cognitive dysfunction includes executive-related disorders associated with apathy, attention deficit memory, difficulties in memorizing new information, difficulty in extracting information from autobiographical memory without a time gradient, (which is in turn characteristic of Alzheimer's disease) dysfunctional memory and spatial disorders. However, language functions and semantic memory are relatively better preserved.[31] An important feature of HD is a lack of awareness or lack of insight into the nature or severity of symptoms that the patient experiences, despite its visible symptoms. This may include a lack of awareness of any disease characteristic, including all three domains of motor, cognitive or behavioral symptoms.[32]

This feature makes it important to consider family members as helpful (sometimes key) sources of information that provide objective assessments of patient symptoms, function levels, and should be involved in assessing the patient's health and decision making. Neuropsychiatric symptoms in HD may precede the onset of motor disorders even up to 15 years before full-blown disease, which contributes to delaying the proper diagnosis of the disease, especially in the absence of a positive history of HD or treatment of a completely different disease (e.g. depression or schizophrenia).

Of particular interest is the juvenile form of the disease (JHD), with a large number of CAG repeats. The first symptoms occur before the age of 20.[33] This form affects 5-10% of carriers of the mutant gene, and only 1% of symptoms occur before the age of 10. JHD is characterized by a difference, but above all a variety of clinical symptoms, especially in the initial stage of the disease. In the case of a negative family history, it makes it difficult and definitely delay the time of making the correct diagnosis. The parkinsonian syndrome (stiffness and bradykinesis) dominates among movement disorders, not chorea. In the Westphal variant, dystonia, ataxia, dysarthria and pyramidal symptoms are observed more often than in the classical form. Mood and behavior disorders are the first symptom of the juvenile form of Huntington's disease in almost 1/3 of patients. The spectrum of disorders is very wide: from irritability, impulsiveness, depressed mood through addiction to alcohol and drugs. It may lead to psychotic states with aggressive behaviors and depressions with thoughts and suicidal attempts, which often require hospitalization in psychiatric wards.[34] Depression and suicide are a significant problem in this population, especially if they have witnessed a family member's illness.[35] Death occurs on average after 8-10 years from the first symptoms.

HD treatment

Currently, Huntington's disease is incurable, there are no effective treatments - it leads to disability and total dependence on care.

Current treatment of HD is often symptomatic and focuses in decreasing dysarthria, dystonia, swallowing complication, incontinence as well as psychological problems and irritability. Therapy for the disease and co-morbid psychiatric symptoms (psychosis or bipolar disorder, episodic aggression and agitation not managed by behavioral interventions) could be treated better by neuroleptic medications. Some patients need raising doses of anti-chorea medications over time, hence re-evaluation of therapy is advised. Some other patients may develop increased dystonia and rigidity in parallel with HD progression, thus reducing the anti-chorea medicines may be crucial. In 2008, tetrabenazine became the first drug for HD, approved in the US, due to its important reduction of chorea determined by the double-blind placebo controlled TETRA-HD study. However patients should be aware of some side effects that may occur, before treatment begins.[36] HD often leaves patients without good treatment options. The lack of treatment options combined with the inevitably deadly nature of the disease contributes to a suicide rate of 5 to 10 times higher in HD patients than in the general population.[37]

Typical course of HD

A typical person with HD gets to know about the condition when a parent is diagnosed with the disease. It happens at the average age of 15, then the person often discusses or attempts suicide. At the age of 16, he or she knows that there is a 50% risk of inheriting the neurodegenerative disease. At the age of 25 (usually already married), predictive DNA testing begins and he or she learns about the inherited mutant HD gene. Two years later, after a prenatal test result is negative for the disease, a woman may have her first child born. At the average age of 34 years, he or she may be diagnosed with beginning of cognitive and motor signs of the disease. In the next 5 years, he or she may be dysfunctional at work and can experience the death of her affected parent. At the average age of 46, a person may be placed in a long-term care facility for 24-hour assistance. The typical person with HD dies in her early 50s. The mutant genes display the same phenotype as heterozygotes, and the phenomenon of "anticipation".[38]

The unique nature of Huntington's disease

The unique nature of HD results from its inheritance. The disease in the family extends over several generations. Every child who has an HD parent has a 50:50 chance of inheriting the faulty gene. Diagnostic and preclinical tests that have been performed since 1993, confirm unambiguously whether a person is ill or not. In every person who has the result of additions, will definitely develop HD, hence such fears in potential patients before performing the genetic test.[39] However, a small number of potentially endangered people are deciding to implement them. The disease disrupts the entire family system. It appears at the moment of the greatest life stabilization, when the most important decisions related to even having children are taken, when the next generations - grandchildren - exist. This situation cannot be reversed. HD does not disappear with the death of the patient, as is the case in other diseases. All children may get sick and each of them may have a different form and other clinical symptoms.

There is always a change in relationships within a family. A marriage may break up because of the blame on the spouse for transferring the gene.

In HD, there is also a unique impact of the disease on family caregivers, who often take care of several generations of patients (parent, spouse, siblings, children). They are afraid about getting sick or that HD may develop in other family members. An additional burden is the tension due to the decision to pass this information to children and other family members.[40] The disease is hidden in the family, it is not discussed on a daily basis, but it is always present in it. It is hidden for fear of social stigmatization or discrimination.[41]

The carrier "silently observes" subsequent family members in terms of possible symptoms and effects of HD, and this is very difficult due to the variety of disease symptoms.

Conclusion

The unique nature of the disease and the complex problems of patients and their families are a challenge for healthcare professionals. HD is a rare disease and employees of both medical and social assistance do not have experience working with people and families affected by HD. They are not always prepared to solve problems resulting from the complex dynamics of change in families with Huntington's disease, from multigenerational disease. You should also pay attention to family carriers, they bear the main burden of caring for the sick in the family.

In our country since 2002, there is the Polish Huntington's Disease Association, which associates people with HD, their carriers and relatives as well as doctors and medical staff (http://www.huntington.pl).

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.12

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 175–178

REVIEW PAPER

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Glycosaminoglycan concentration in cancer tissue

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ABSTRACT

Introduction. Glycosaminoglycans (GAGs) play a widespread role in tissue modelling. GAG polymers may affect several receptor pathways in parallel.

Aim. To present difference in concentration of GAG in healthy and cancer tissues.

Material and methods. The literature search was performed nd reviewed using selected keywords.

Results. We revieved the methods of detection various types of glycans measured by Magnetic Resonance Imaging.

Conclusion. MRI methodology provides an efficient tool forstudy of cellular composition. The use T_1 aof T_2 measurements to study cancer tissue is a promising assay.

Keywords. fixed charge density, glycosaminoglycan, magnetic resonance imaging

Introduction

Proteoglycans (PG) are one of the major components of the extracellular matrix (ECM). ECM conteinat at least one glycosaminoglycan (GAG) chain such as heparan sulfate, chondroitin sulfate, keratan sulfate, and heparin. PGs are formed of GAGs covalently attached to the core proteins. PG are cellular, subcellular, intracellular, cell surface, pericellular, and extracellular.[1-2] PG are major components of extracellular matrix playing key roles in its structural organization and cell signaling contributing to the control of numerous normal and pathological processes.[3-9] GAG expression occurs in most hematological malignancies, notably acute myeloid leukemia, myeloproliferative neoplasms, and multiple myeloma. Here, we review recent research advances regarding cellular GAG and possible magnetic resonance applications to measure GAG concentrations.

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 29.10.2018 | Accepted: 29.11.2018
Publication date: June 2019

Bar P, Galiniak S, Bartusik-Aebisher D, Filip R, Aebisher D. *Glycosaminoglycan concentration in cancer tissue.* Eur J Clin Exp Med. 2019;17(2):175–178. doi: 10.15584/ejcem.2019.2.12

Magnetic Resonance Imaging and celluar GAG measurements

Relaxation times measuremens (T_1 and T_2) of cancer tissue protons can be determined by Magnetic Resonance Imaging (MRI). Because of the development of MRI methods many cellular properties of tumor tissue is studied with MRI. MRI relaxation times have been shown to be various for many types of tumors compared to normal tissues. MRI is an important non-surgical tool in medical and biomedical analysis. While standard MRI can provide basic information regarding tumor location, its size and spread, the quantified MRI can evaluate the effectiveness of therapy. It was already shown that treatment of cells results in MR contrast changes due to changes in relaxivity caused by cell shrinkage and cellular membrane blabbing. We contribute changes in T_1 and T_2 during the cell growth observed for both cell lines to the changes in tissue hydratation and protein content. In addition, our study showed that proton T_1 and T_2 relaxation times are not significantly different between both cell lines.

Glycosaminoglycan (GAG) [9-24]

The GAG concentration was calculated based on Fixed Charge Density (FCD) value, which was measured by flushing the culture with $Gd(DTPA)^{2-}$. The FCD can be expressed as:

$$FCD_{tissue} = -2\left[Na^+\right]_{bath}\left(\sqrt{\frac{\left[Gd(DTPA)^{2-}\right]_{tissue}}{\left[Gd(DTPA)^{2-}\right]_{bath}}} - \sqrt{\frac{\left[Gd(DTPA)^{2-}\right]_{bath}}{\left[Gd(DTPA)^{2-}\right]_{tissue}}}\right)$$

{Eq. 1}

Where:

$$\left[Gd(GDPA)^{2-}\right]_{tissue} = \frac{1}{R}\left(\frac{1}{(postGd)T_{1(tissue)}} - \frac{1}{(preGd)T_{1(tissue)}}\right)$$

{Eq. 1a}

and

$$\left[Gd(DTPA)^{2-}\right]_{bath} = \frac{1}{R}\left(\frac{1}{(postGd)T_{1(bath)}} - \frac{1}{(preGd)T_{1(bath)}}\right)$$

{Eq. 1b}

Where:

Bath – medium around the breast cancer cells;

R – relaxivity (mmol/L/sec);

Tissue – breast cancer cells tissue;

$[Na^+]_{bath}$ – concentration of Na^+ ions in bath, 154 (mmol/L);

$(postGd)T_{1(tissue)}$ – T_1 relaxation time of the breast cancer cells after administration $Gd(DTPA)^{2-}$ solution in sec;

$T_{1(tissue)}$ – T_1 relaxation time of the breast cancer cells before administration $Gd(DTPA)^{2-}$ solution in sec;

$(postGd)T_{1(bath)}$ – T_1 relaxation time of the bath after administration $Gd(DTPA)^{2-}$ solution in sec;

$T_{1(bath)}$ – T_1 relaxation time of bath before administration $Gd(DTPA)^{2-}$ solution in sec.

The calculated FCD is converted to GAG concentration according equation 2:

$$GAG = FCD\left(\frac{502.5}{-2}\right)$$ *{Eq. 2}*

Where: *GAG*- Glycosoaminoglycan concentration (mg/L);

FCD – Fixed Charge Density (mmol/L);

502.5 – Molecular weight of GAG in (mg/mmol).

Application of enables direct study of cells before and after treatment. The T_1 and T2 relaxation time of cells is sensitive to GAG concentration. Therefore, MRI measurements of cells with the use of anionic paramagnetic contrast agent $Gd(DTPA)^{2-}$ reflect directly to the GAG concentration in tissue and is sensitive to physiologic and pathologic conditions resulting in an approximately linear relation between GAG content and T_1 relaxation time. Since GAGs have negatively charged side chains, the $Gd(DTPA)^{2-}$ distributes in higher concentration into areas with lower GAG concentrations. Therefore, a low T_1 values after contrast agent administration indicates low GAG concentration.

In oncology non-invasive imaging of cells has gained interest for the assessment of tumor response to cancer therapy. Therefore, MRI has become an important diagnostic technique for characterization of cells, such as degeneration. Due to variability in response to therapy, there is a growing interest in monitoring efficacy progress during treatment. There is a rapid increase in the applications of MRI for cellular imaging. Table 1 presents selected types of cellular PG's.

Eponym	Secretory granules	Location
Serglycin[24]	Transmembrane	Cell surface
Syndecan[25,26]	Transmembrane	Cell surface
NG2[27,28]	Transmembrane	Cell surface
Betaglycan[29,30,31]	Transmembrane	Cell surface
Phosphacan[32,33]	Transmembrane	Cell surface
Glypican[34,35]	Glypican	Cell surface
Perlecan[36]	Basement membrane zone	Pericellular
Agrin[37]	Basement membrane zone	Pericellular
Aggrecan[38]	Hyalectan Lectican	Extracellular
Versican [39]	Hyalectan Lectican	Extracellular
Neurocan[40]	Hyalectan Lectican	Extracellular
Brevican[41]	Hyalectan Lectican	Extracellular

Conclusion

MRI methodology provides an efficient tool forstudy of cellular composition.The use T_1 aof T_2 measurements to study cancer tissue is a promising assay.

Acknowledgments

Dorota Bartusik-Aebisher acknowledges support from the National Center of Science NCN (New drug delivery systems-MRI study, Grant OPUS-13 number 2017/25/B/ST4/02481).

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© Wydawnictwo UR 2019
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.13

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 179–183

CASUISTIC PAPER

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Acute pulmonary hypertension as a symptom of Bard's syndrome and pulmonary lymphangitis carcinomatosa – rare manifestation of malignant gastric cancer

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ABSTRACT

Introduction. Acute pulmonary hypertension leading to right ventricular failure and circulatory collapse is usually caused by a pulmonary embolism. However, in extremely rare cases, similar clinical manifestations can be related to another diseases, such as lymphangitis carcinomatosa.

Aim. The purpose of this paper is to report on the case of a 29-year-old male patient presented with rapidly progressing dyspnoea.

Description of the case. The diagnosis of pulmonary embolism was made on the basis of echocardiographic signs of pulmonary hypertension and right ventricular (RV) dilatation, and the recommended therapy was introduced. On the suspicion of bronchopneumonia, antibiotics and steroids were applied. However, the previously stated diagnosis of pulmonary embolism was not confirmed by the angio-CT scan, which showed small diffusive lung parenchyma intra-biliary nodules (ground glass opacity) with the peripheral appearance of a tree-in-bud sign. Consecutive CT of pelvis and abdomen along with endoscopy revealed a metastatic gastric cancer with the presence of lymphangitis carcinomatosa and miliary dissemination to the lungs. The presence of pulmonary metastases in the course of disseminated gastric cancer is known in literature as Bard's syndrome.

Conclusion. Extrapulmonary malignancies, particularly gastric cancer, should be taken into consideration in differential diagnosis in patients with an acute right ventricular failure and nonspecific lesions in the respiratory system.

Keywords. Bard's syndrome, malignant gastric cancer, pulmonary hypertension

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 7.03.2019 | Accepted: 28.03.2019
Publication date: June 2019

Mazur D, Romanek J, Filip R, Przybylski A. *Acute pulmonary hypertension as a symptom of Bard's syndrome and pulmonary lymphangitis carcinomatosa – rare manifestation of malignant gastric cancer*. Eur J Clin Exp Med. 2019;17(2):179–183. doi: 10.15584/ejcem.2019.2.13

Introduction

Acute right ventricular (RV) failure can be defined, as a rapidly progressive syndrome with a systemic congestion, resulting from an impaired RV filling, and/or reduced RV flow output.[1,2] Most often, it is associated with increased RV afterload, or preload and consequently RV chamber dilatation, and tricuspid regurgitation.[3,4] The most common causes of acute RV failure are: acute left ventricular failure, right ventricular ischaemia, acute pulmonary embolism, and exacerbation of chronic lung disease.[1] Described pathology is observed in 3% to 9% of acute heart failure admissions, and in-hospital mortality of patients with acute RV failure; ranges from 5 to 17%.[1]

According to Global Cancer Statistics 2018: GLOBOCAN stomach cancer remains an important cancer worldwide: is the fifth most frequently diagnosed cancer, and the third leading cause of cancer death.[5] Distant metastases of this tumor are located in lungs twice more frequently among males.[5] Military metastases of adenocarcinoma of the stomach, called also as Bard's syndrome, were described by Samson et al. at 1962.[6] Apart from single, or multiple tumors in the parenchyma of the lungs, metastatic lesions may manifest themselves as the lymphangitis carcinomatosa, and the pleuritis carcinomatosa.[7] These kinds of metastases of gastric cancer share the feature, that the symptoms in the respiratory system dominate over the symptoms in the alimentary tract.[7]

Description of the case

A 29-year-old patient without past comorbidities, was admitted to the Emergency Unit (EU) with dyspnoea, dry cough, shortness of breath, and reduced exercise tolerance. Such complaints occurred three weeks ago and increased over two days.

Examination revealed: pallor, tachycardia (110 bpm), tachypnoea (breathing rate average 30/min) with oxygen saturation of 92% and BP 110/70mmHg. The respiratory examination provided evidence of crackles at the base of both lungs. The ECG record was normal. The laboratory tests revealed significant D-dimer elevation with slightly increased level of troponin and CRP (Table 1). Symptomatic treatment was introduced: hydrocortisone i.v., fenoterol and ipratropium in nebulisation, without improvement of patient's condition.

Due to D-dimer increment transthoracic echocardiography (TTE) was performed. The following abnormalities occurred: right ventricle overload (RVd=34mm) RV>LV, ACT on the pulmonary valve 78 m/s and TRPG 35mmHg, with moderate tricuspid valve regurgitation (VC=5mm), TAPSE 18mm. According to the 2014 ESC Guidelines, on the diagnosis and management of acute pulmonary embolism, the CT angiography of pulmonary arteries was requested. Meanwhile, the patient had been admitted to the Intensive Care Unit of Cardiology Department with preliminary diagnosis of not high-risk pulmonary embolism, and echocardiographic symptoms of acute pulmonary hypertension.

The CT angiography of pulmonary arteries revealed widening of the pulmonary trunk (approx. 28.5 mm), and arteries (left-23 and right-22mm). Narrow contrast deficits were observed only in more peripheral pulmonary arteries at the level of the lower lobes.

Furthermore, the results of the CT scan presented diffused, small intralobular nodules (ground glass opacity) in lung parenchyma with the peripheral appearance of a tree-in-bud sign. The presence of nonspecific findings in the lower lobes, directed differential diagnosis of allergic alveolitis, interstitial disease, or others. The notes included also several abnormalities such as: the slightly enlarged lymph nodes in the lung cavities, enlarged sub-nodal nodes, the right lower lobe bronchus and lymph nodes in the mesenteric fat tissue, up to 15 mm.

The treatment was enriched by low molecular weight heparin injections in therapeutic doses, fluids and supplemental oxygen with a slight improvement of patient's condition.

The imaging examination with ultrasonography of lower limbs veins, thyroid gland and abdomen was established. Following pathological findings were described: asymmetrical widening of gastric antrum, cervical lymphadenopathy, a small normoechogenic tumor in the left lobe of the thyroid. It needs to be mentioned: viral markers, including HIV, HBV and HCV, EBV, cytomegaloviruses, were non-reactive for serology. Other scans appeared to be negative for atypical infections such as: *Mycoplasma pneumoniae*, *Chlamydophilia pneumoniae*, and *Legionella pneumophilia*. In addition to that, blood and sputum cultures, cancer markers as CA 125, CA19-9, were negative as well.

Other, more relevant laboratory tests results, are presented in the Table 1.

Table 1. Laboratory results of patient

	At the day of admission	Fifth day of hospitalization
CRP mg/l [0.0–5.0]	29.3	17.6
WBC 10^3/µl [4.00–11.00]	8.23	14.6
D-DIMER ng/ml [0–500]	14300	3795
NT-proBNP pg/ml [0–125]	409	5444
TNThs pq/ml [3.0–1.4]	90.32	31.01

The patient's condition improved slightly after the symptomatic treatment composed of inhaled bronchodilators, intravenous steroids, diuretics. Moreover, wide spectrum antibiotics were administered intravenously. On the fourth day of hospitalization, the patient's condition deteriorated, and sudden respiratory and cardiac arrest occurred. Resuscitation activities (CPR) were successful. Full consciousness returned however symptoms

of hypoxemia (oxygen saturation up to 70%), and tachycardia (150/bpm) remained.

The TTE presented significant right-sided ventricular overload, with features of severe pulmonary hypertension, and tricuspid valve gradient up to 90mmHg (Fig. 1, 2).

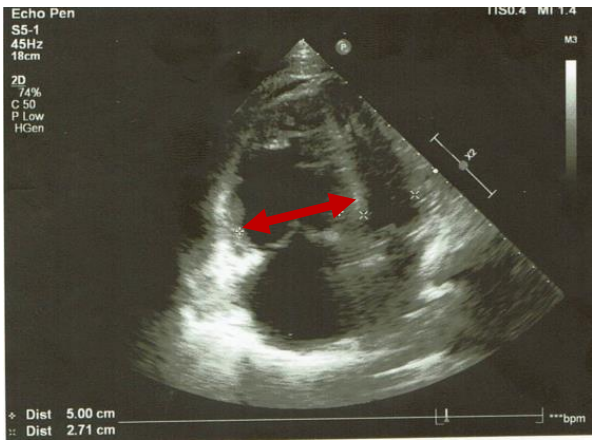

Fig. 1. Right ventricular dilatation with McConnel sign

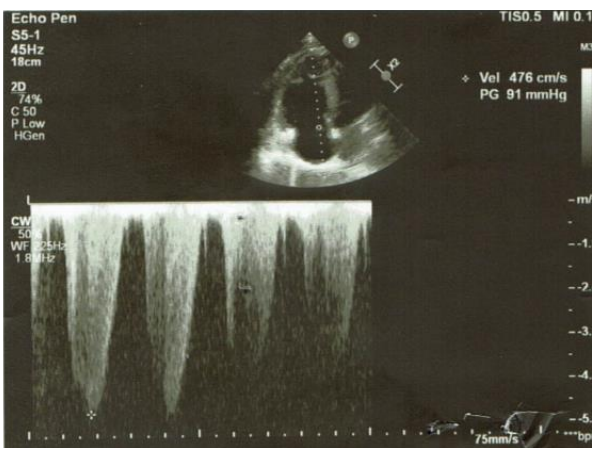

Fig. 2. Tricuspid regurgitation peak gradient (TRPG) 90mmHg

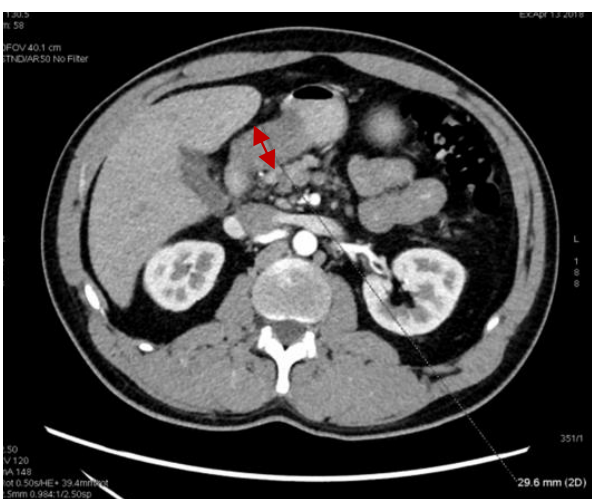

Fig. 3. Circular infiltration of gastric antrum

Repeated chest angiography revealed widening of the pulmonary trunk up to 31mm, without central or peripheral embolization.

Further diagnostic process, enriched by abdomen and pelvis contrast CT, confirmed pathological findings in the area of the gastric antrum (Fig. 3).

The diffused lymphangitis was found in the following areas: the antrum (18mm), pylorus (15mm), lesser curvature of the stomach, hepatic hilum, mesentery, near the celiac trunk and aorta (13mm).

Gastroscopy uncovered gastric mucosa infiltrations in the area of greater curvature of stomach. Histopathologic study of preantral part of stomach demonstrated poorly cohesive carcinoma, with inclusion of signet ring cell carcinoma. (Fig. 4)

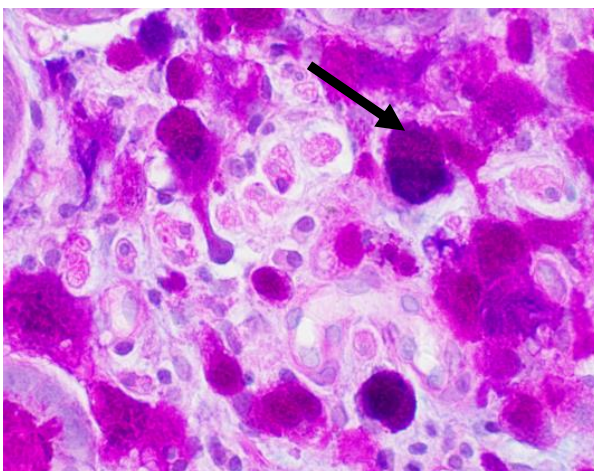

Fig. 4. Signet-ring carcinoma cell – laterally displaced nucleus by a cytoplasmic vacuole. Alcian blue staining.

On the sixth day of hospitalization, a respiratory and a cardiac arrest occurred. The resuscitation was unsuccessful.

The diagnosis was established based on the clinical presentation and investigations.

The cause of the death was cardiogenic shock due to acute RV failure, as a consequence of poorly differentiated carcinoma of the stomach with diffuse pulmonary metastasis, and lymphangitic carcinomatosis.

Discussion

The term pulmonary lymphangitic carcinomatosis (PLC) was first coined by Troisier in 1873 to describe a condition of diffuse infiltration of the lymphatics of both the lungs by malignant cells.[8] The analysis of literature data performed by Bhattacharya et al. relates to such condition with greater predilection for males (60:40), and has a greater propensity to younger population.[8,9] The most common locations of the primary tumor are: lungs, breasts, pancreas, stomach and prostate.[8,10] Li Zhuang explains the pathologic features of PLC as a consequence of primary liver cancer.[11] Patho-

physiologically, cancer in the mediastinal and pulmonary hilar lymph nodes may obstruct lymphatic drainage, resulting in retrograde migration of cancer cells into terminal lung tissues via lymphatic vessels, or anterograde migration of cancer cells in the pleura into the pulmonary hilar lymph nodes through intrapulmonary lymph vessels.[11,12]

Poorly differentiated adenocarcinomas, and signet-ring cell carcinomas found in the intra hist-pat study, were classified as "undifferentiated histological types", according to the Japanese classification of gastric carcinoma.[13] Such malignancy with invasion into deep penetration into the submucosa, lymphatics or venules is associated with an increased risk of nodal metastases.[13] Signet ring histology is an independent predictor of poor prognosis in gastric adenocarcinoma.[6,14]

Interstitial lung disease (ILD) encompasses a large, and diverse group of pathological conditions that share similar clinical, radiological and pathological manifestations with different aetiologies.[15] Results of CT scans, described as the ground glass with a tree-in-bud sign, can be interpreted as: intensive inflammation various in degree, and fibrosis of an interstitial tissue of the lungs or very rarely miliary neoplastic pulmonary metastases.[7]

On the microscopic level, such metastases are characterized by the presence of single neoplastic cells or their clusters in the lumen of blood and lymphatic vessels of the lungs sometimes accompanied by thrombi consisting of thrombin and platelets.[7] Pathophysiological lesions lead to constriction of the vessels lumen, thus leading to perfusion disorders of the large area of the lungs with further disorders of the perfusion to ventilation ratio.[7]

In the presented case, narrow contrast deficits were observed only in more peripheral pulmonary arteries in CT scan on the day of admission. The second CT scan did not reveal microembolisms. Moreover, discovered significant decrease of D-dimer, probably, was a result of the heparin treatment, but it was not associated with a clinical improvement.

According to Murry et al. disseminated pulmonary tumor embolism should be suspected in a patient with cancer who has dyspnoea, hypoxemia, and unexplained PH, and is a common autopsy finding in patients with cancer, but it is rarely diagnosed premortem.[16] Contrast-enhanced CT, and pulmonary angiography are not helpful to detect microembolisms, and their negative predictive value is low.[16] In order to confirm the diagnosis of microscopic pulmonary tumor embolism, tissue must be obtained by either open-lung or transbronchial lung biopsy.[17]

Such a method was refused due to terminal condition of the patient. However, based on the progression of the disease and the described results of the imaging, reference can be made to those with a related course.

Treatment of low-density heparin was included due to primary suspicion of pulmonary embolism based on radiological, and echocardiographic examination. Dexamethasone, and antibiotics were added as the treatment method of systemic connective tissue disease and bronchopneumonia. Thus, surgical treatment remains the treatment of choice for early stage signet ring carciomas.[18]

Currently, there are no proven effective treatment strategies for PLC.[9] Based on recent studies chemotherapy with a regimen of oxaliplatin, leucovorin and 5-fluorouracil, did not lead to life extension in the case described by Gilchrist et al.[19] Despite that, there are case reports of platinum-based chemotherapy leading to transient remission.[20]

Patients with PLC included in the study of Densteed et al. (six patients with an average age 26 and primary gastric tumor), had a mean survival time of 22 days after their first admission to hospital.[19]

Conclusion

Poorly differentiated cancer is believed to show poor prognosis and aggressive behavior. Presented manifestation of malignant gastric cancer with abrupt progression, RV failure with symptoms of pulmonary hypertension, can be a consequence of respiratory and lymphatic systems affection by metastases.

Treatment options in high severity are limited and not associated with life extension.

Followed conclusions can be made after analysis of similar, described in literature cases, associated with the microembolisms or pulmonary lymphangitis carcinomatosa or heterogenous lung metastases.

Finally, extrapulmonary malignancies should be considered in patients with symptoms of acute right ventricular failure and nonspecific lesions in the respiratory system.

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ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2019.2.14

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2019; 17 (2): 184–191

CASUISTIC PAPER

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Fibrodysplasia Ossificans Progressiva – a presentation of cases and literature review

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ABSTRACT

Introduction. Fibrodysplasia ossificans progressiva (FOP) is a very rare inherited disease leading to progressive ectopic ossification of muscle and soft tissue and resulting in severe immobilisation and premature death. The mutations in *ACVR1* gene that codes the 1A activin receptor which belongs to the family of bone morphogenetic proteins (BMPs) are leading to clinical symptoms.

Aim. In this raport we present 3 cases of paediatric FOP patients presenting varied clinical course of disease.

Description of the cases.

Case 1. A girl, currently 5 years old, was hospitalised for the first time at the age of 10 months with suspicion of a hyperplastic lesion of the left lumbar area. The time period between the first symptom, i.e. subcutaneous oedema, and the correct diagnosis was about 8 months. The symptom with key importance for the diagnosis was congenital deformities of the thumbs and big toes.

Case 2. A 6-year-old girl with a congenital hallux valgus in both feet, a small painless nodular lesion in the area of the distal metaphysis of the femur, limiting the flexion of the knee joint, was diagnosed in the third month of life.

Case 3. A three-year-old girl was diagnosed with congenital defects i.e. hallux valgus of both feet. The first symptoms of the disease occurred during her 14th month when an oedema of the subcutaneous tissue of the nape area was observed.

Conclusion. Until recently, there has been no efficient therapy which could slow down the natural course of the disease and currently the disease is treated as incurable. Of key importance from the perspective of patients is early diagnosis and, more importantly, preventing traumas, surgical procedures, intramuscular injections, sparing dental treatment and ensuring avoidance of airway tract infections.

Keywords. clinical course, diagnostic difficulties, ectopic ossification, fibrodysplasia ossificans progressiva

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 25.02.2019 | Accepted: 1.05.2019
Publication date: June 2019

Dąbrowska M, Dąbrowski P, Tabarkiewicz J. *Fibrodysplasia Ossificans Progressiva – a presentation of cases and literature review*. Eur J Clin Exp Med. 2019;17(2):184–191. doi: 10.15584/ejcem.2019.2.14

Introduction

Fibrodysplasia ossificans progressiva (FOP) is a very rare genetic disease leading to progressive ectopic ossification of muscle and soft tissue. This disease occurs irrespective of sex, race or geographical latitude in 1 per 2 million live births.[1,2] The first case was described more than 250 years ago by a London physician, John Freke, in a report from 1740. The next report that focused on FOP was authored by Jules Rosenstirn from San Francisco in 1918.[1]

Fibrodysplasia ossificans progressiva is inherited in an autosomal dominant manner with a low gene penetration. This mutation, discovered in 2006, occurs in the *ACVR1* gene that codes the 1A activin receptor which belongs to the family of bone morphogenetic proteins (BMPs). The 617G> A (R206H) mutation of the 1A activin receptor gene (*ACVR1, ALK2*) is present in the majority of FOP patients and is regarded as disease specific. There have been incidental reports of the mutation of the *de novo* type e.g. in *ACVR* 1,067 G> A gene (G356D). The 1A activin receptor is present in all body tissues including muscle and chondral tissue in which it helps to control development and growth of bones and muscles and ossification of chondral elements.[2, 3] A consequence of this mutation is excess BMP-4 protein produced in white blood cells that is responsible for normal osteogenesis in the fetal stage. Additionally, a low level of the BMP-4 antagonist noggin, which inhibits bone growth and development, is noticed in patients affected with the disease.[4,5] Currently, intensive studies are concerned with the following: an examination of the BMP-signalling pathway, a search for activin receptor inhibitioning proteins, evaluation of toll-like receptor-bone morphogenetic protein (TLR-BMP) signalling, the role of the innate immune system, including monocytes, macrophages, mast cells, natural killer (NK) and dendritic cells as well as T and B lymphocytes and proinflammatory cytokines. The research question posed in these studies is whether FOP is an autoinflammatory or autoimmune disease. The answer to this question will determine the search for efficient treatment methods.[6-8]

The main clinical manifestation of this disease is ectopic ossifications which usually occur in a specific sequence moving vertically downwards in the body, gradually leading to patient immobillity in their 2-3rd decade of life. The outcome of this process is premature death at approximately 40 years of age. Death occurs as a result of circulatory and respiratory insufficiency, frequently complicated with pneumonia.[10,11]

There are no specific tests allowing for a quick diagnosis, apart from the determination of a typical genetic mutation. The symptoms which might be helpful in a correct diagnosis are congenital defects of the toes and fingers.

Description of the cases

Case 1.

A girl, currently 5 years old, was hospitalised for the first time at the age of 10 months with suspicion of a hyperplastic lesion of the left lumbar area. The results of diagnostic laboratory tests were inconclusive. The CT and MRI examinations revealed a well-defined abnormal soft-tissue structure adjacent to the dorsal muscles, measuring 11×94×123 mm, not exceeding the dorsal fascia and not penetrating the spinal canal. The lesion was qualified for surgical resection. The findings from the physical examinations performed one day before the surgery revealed significant growth of the lesion to such an extent that surgery was abandoned. A physical examination, performed at the age of 18 months, revealed a hard oedema of subcutaneous tissue involving the neck, nape, back and the lumbar area and, in the projection of the right scapula – an elevated nodular lesion was observed. The oedema restricted the mobility of the entire spine and right shoulder joint. Only then was particular attention paid to the shortening of the thumbs in both hands and the *hallux valgus*, observed immediately after birth of the child and qualified as a congenital defect of the bone system. The laboratory tests revealed only a slight increase of alkaline phosphatase activity. The imagining diagnostics, comprising an ultrasound and X-ray performed at that stage, did not reveal any significant abnormalities with the exception of extensive oedema of the subcutaneous tissue. The differential diagnostics took into consideration systemic diseases of the connective tissue, fasciitis and oncological diseases of soft tissues. The presence of anatomical anomalies of the thumbs and large toes aided in a correct diagnosis, as on the basis of these symptoms, a suspicion of FOP was noted and a typical *ACVR1* gene mutation was confirmed. The child was subjected to chronic ibuprofen and anti-leukotriene therapy. In periods of exacerbations and after traumas, the treatment also included glucocorticosteroids (GCs). Moreover, for 2 years, the child was treated with pamidronate in intravenous infusions for 3 consecutive days every 3 months. From the time of pamidronate introduction, a significant decrease in the frequency of exacerbations was observed. Despite the applied therapy, within a 3.5 year follow-up period, a significant progression of the disease occurred with numerous ossifications and a silhouette deformity. A significant reduction of the mobility of the entire spine, frozen shoulder and elbow joints, hard palpable lumps and multiple ossifications within the chest (Fig. 1.) and abdominal cavity reaching the iliac wing (Fig. 2.) were observed. An X-ray of the spine revealed ossifications of the cervical vertebrae (Fig. 3.), numerous perispinal ossifications in the thoracic and lumbar spine, massive additional calcifications and ossifications within the nape soft tissues to the level of Th-6. In other areas, surplus

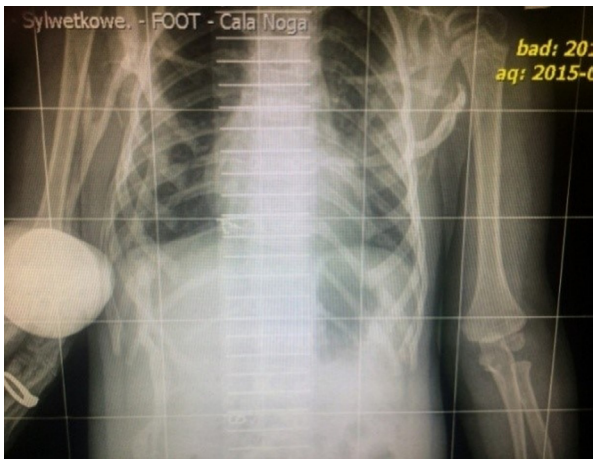

Fig. 1. Chest X-ray examination of Patients 1 showing multiple ossifications within the chest

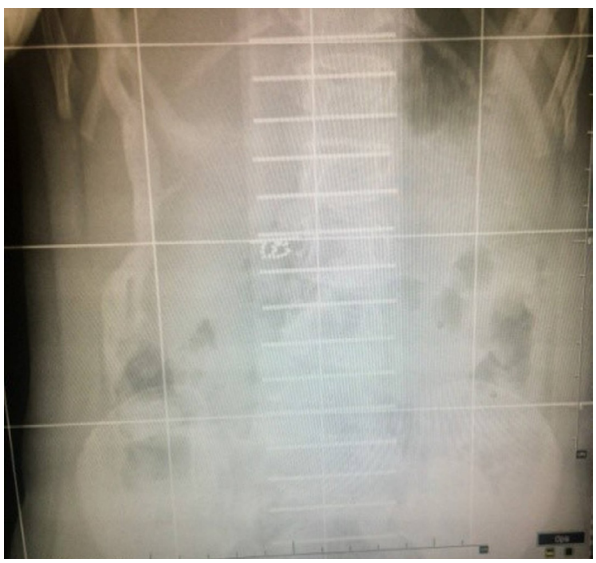

Fig. 2. X-ray examination of Patients 1 showing ossification in abdominal wall reaching the iliac wing

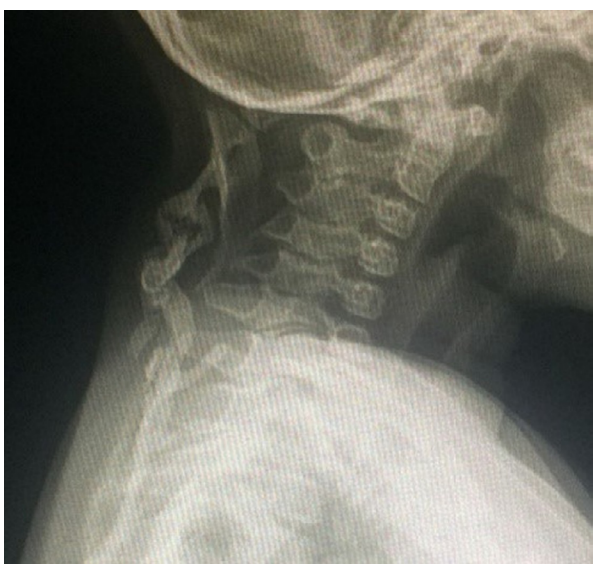

Fig. 3. X-ray examination of Patients 1 showing ossifications of the cervical vertebrae

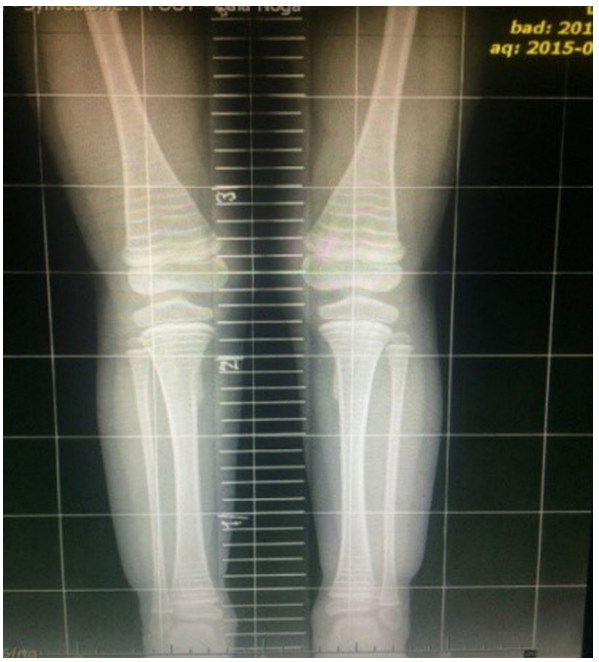

Fig. 4. X-ray examination of Patients 1 showing surplus bone in both proximal tibial metaphyses

bone in both proximal tibial metaphyses (Fig. 4.) and bilateral deformities with widening and shortening of the femur were found. The cause of the last hospitalization was diffuse hard nodules in the subcutaneous tissue reaching from the mandibula to the manubrium of the sternum which occurred during an infection of the upper respiratory tract. The treatment was comprised of pulses of methyl-prednisolone leading to a fast regression of the lesions. In the described case, the time period between the first symptom, i.e. subcutaneous oedema, and the correct diagnosis was about 8 months. The symptom with key importance for the diagnosis was congenital deformities of the thumbs and big toes.

Case 2.

A 6-year-old girl with a congenital hallux valgus in both feet, a small painless nodular lesion in the area of the distal metaphysis of the femur, limiting the flexion of the knee joint, was diagnosed in the third month of life. On the basis of a typical X-ray image, a chondro-osteophyte was diagnosed, which was then surgically removed at the age of 11 months. In the post-operative period, a formation of hematoma was observed in the scar, which then limited the mobility of the joint. Within a four-month follow-up period, the lesion had increased 2-3 fold. The diagnosis was of a recurrence of the chondro-osteophyte, measuring then 3.4 cm × 2.4 cm with adjacent calcifications. The child underwent revision surgery. The histopathological report described alteration of the chondro-osteophyte morphology with hyaline cartilage and a wide band of enchondral ossification; under a connective tissue layer there was also

a band of metaplastic myxoidal cartilage. Three weeks after the revision surgery, a painful and tender oedema of the scar reaching the proximal part of the femur was observed and, after another 6 months, a follow-up X-ray revealed some other numerous chondro-osteophytes along the entire length of the scar on the medial side of the proximal segments of both tibias. Another surgery was proposed, but the parents did not consent. Two and half years after the first symptom, on the basis of a clinical picture, and on the current big toes anomaly, a suspicion of FOP was made. The presence of a heterozygotic p.R206h mutation in the sequence coding the *ACVR1* gene was dertermined in a genetic examination. Currently, in the physical examination, some shortening and *hallux valgus* of both big toes was found (Fig. 5); moreover, on the lateral side of the left femur under the post-operative scar, some irregular bone masses reaching the mid-thigh and an immobilisation of the left knee joint with a progressive hyper-extension, and thickening of the medial surfaces of both tibias was found. The imaging diagnostics revealed band-like calcifications in the iliotibial band with a length of approx. 40-50 mm which, segmentally, was connected with the bone, stretching mainly in the lateral and medial vastus muscle; the calcifications in the deep surface of the tendon reached the suprapatellar recess. In the distal part of the left femur, there was an irregularly shaped chondro-osteophyte on a wide base, originating from its antero-lateral surface, along a segment of 7.5 cm long as well as calcifications in the soft tissues (Fig. 6, 7). The patient's treatment comprised a chronic application of indomethacin. After one of the many episodes of falls, causing long-term immobilisations, prednisone was included in the therapy, and after its introduction no additional ossifications were observed.

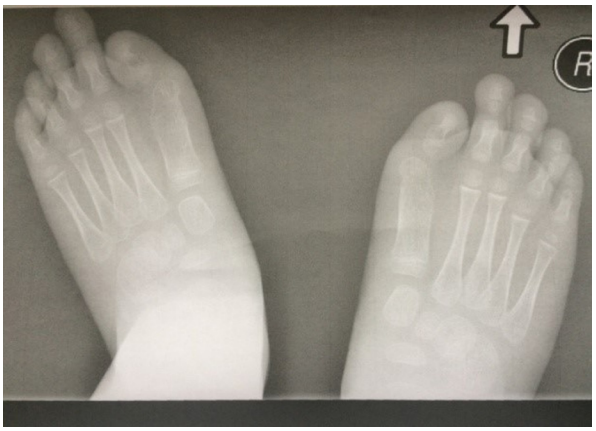

Fig. 5. X-ray examination of Patients 2 showing shortening and hallux valgus of both big toes

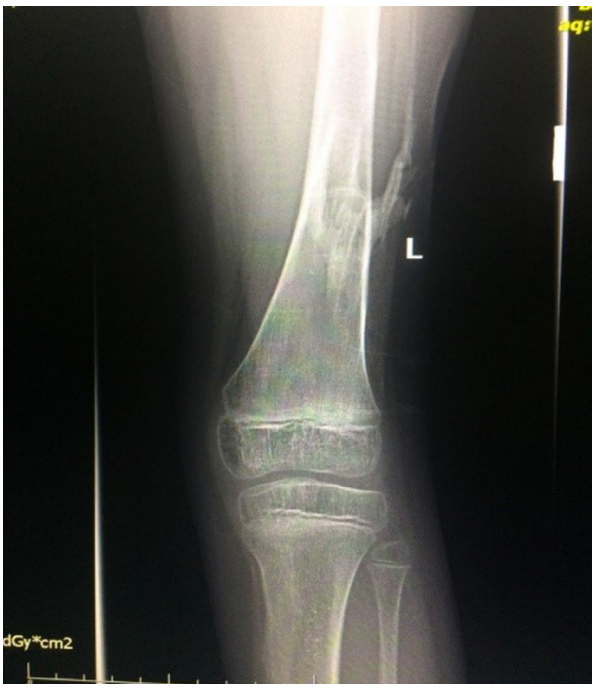

Fig. 6. X-ray examination of Patients 2 showing ossifications in distal part of the left femur

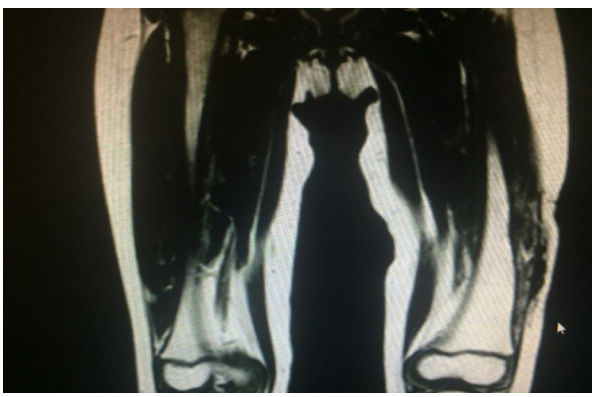

Fig. 7. MRI scan of Patients 2 showing ossifications and calcifications in the soft tissues

Case 3.

A three-year-old girl born from first gestation, with an uneventful family history, was diagnosed with congenital defects i.e. hallux valgus of both feet. The first symptoms of the disease occurred during her 14th month when an oedema of the subcutaneous tissue of the nape area was observed. Immobilisation in a Schanz collar brace and NSAIDs were applied in the therapy. Two weeks after the onset of treatment, the lesion had significantly increased. A physical examination revealed a significant limitation in cervical spine and shoulder joint mobility, as well as a massive oedema covering the nape, neck and the chest, reaching down to the lumbar area. Additionally, an inflammation of the medium lobe of the right lung was diagnosed. The laboratory tests showed an increase in inflammatory protein concentration and an increase in alkaline phosphatase activity. Imaging diagnostics (X-ray and CT) did not show any abnormalities with the exception of the subcutaneous oedema. The ap-

plied treatment was comprised of parenteral antibiotics and ibuprofen. The picture of the disease at that time allowed for diagnosis of FOP, which was confirmed by the presence of the heterozygotic p.R206h mutation in the sequence coding of the A*CVR1* gene. Further observation of the child revealed frequent exacerbations, not only after traumas or infections, but also spontaneous ones, consisting of repetitive episodes of soft tissue oedemas followed by consecutive ossifications of the areas of the nape, neck, scapula, chest and both upper extremities (Fig. 8, 9). A physical examination at the age of 20 months, revealed an antero-flexion of the trunk with a limitation of cervical spine mobility, alleviation of the mobility in the shoulder joints and a limitation of flexion to 90 degrees. Moreover, hard nodular infiltrations and additional ossifications in the frontal, suprascapular and intrascapular areas, on the nape, as well as along the thoracic spine, the left lumbar area, and along the arms and forearms in addition to shortening and valgus of the toes and discreet shortening of both thumbs were observed. The chest and extremities X-ray revealed calcifications and ossifications within the soft tissues, and along the humeral bones joining the chest in the upper medial section of the back and nape. The treatment consisted of chronic administration of the NSAIDs, antileukotriene agents and D3 vitamin supplementation. After episodes of disease exacerbations before dental and laryngological interventions, prednisone was administered. Within the next few months, the dynamics of the occurrence of the lesions was exceptionally pronounced. Numerous exacerbations of the disease were observed which led to the necessity of frequent use of prednisone. At the age of 2.5 years, the girl lost the ability to walk independently and a physical examination showed additionally a hard oedema in the subcutaneous right lumbar area descending to the area of the buttocks and into the proximal part of the right thigh. Laboratory tests revealed increased levels of alkaline phosphatase with other results being within the normal range. An X-ray revealed calcium-saturated bands in the soft tissues of the upper part of the lumbar spine, the area of the buttocks and the sacrococcygeal area and deformities of the necks of both femur and the head of the left femur (Fig. 10). With regard to gait limitation, a decision was taken to administer GCs in the form of Solu-Medrol pulses, according to the 1-3-5 day regime, which allowed for a very rapid improvement of the patient's condition shortly after the first dose of the drug. The girl started to walk the following day. Yet, still some repetitive "flashes" were observed leading to oedemas both in the subcutaneous level of the chest and in the abdomen and extremities, which resulted in the necessity of periodic use of prednisone, ranging from 4 to 5 times per month. At that time, a decision to introduce pamidronate was made. After the administration of the drug, within a 6-month follow-up period, a significant reduction of disease progression was observed. In this case, despite of the quick diagnosis, the lack of effective treatment resulted in continual progress of the disease and consequent disability. The fulminant course of the disease, with a limitation of the mobility of the entire spine, chest, upper and lower limbs, occurring in such a short period of time, are indicative of an extremely aggressive course of the disease.

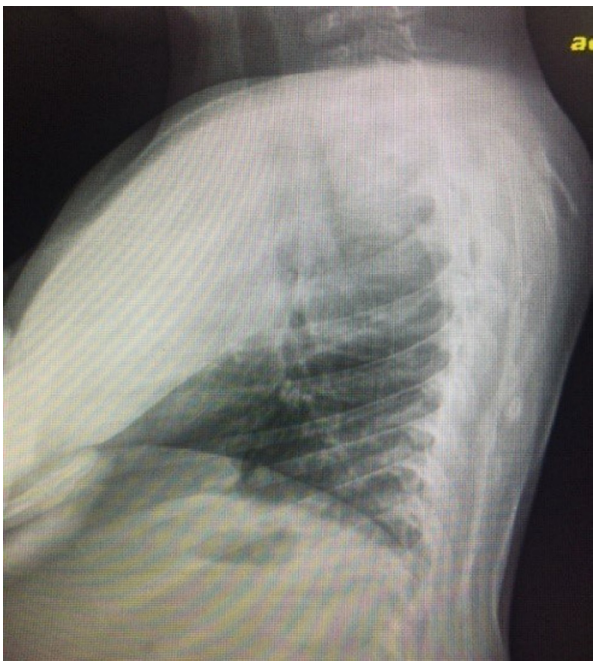

Fig. 8. X-ray examination of Patients 3 showing ossifications in trunk area

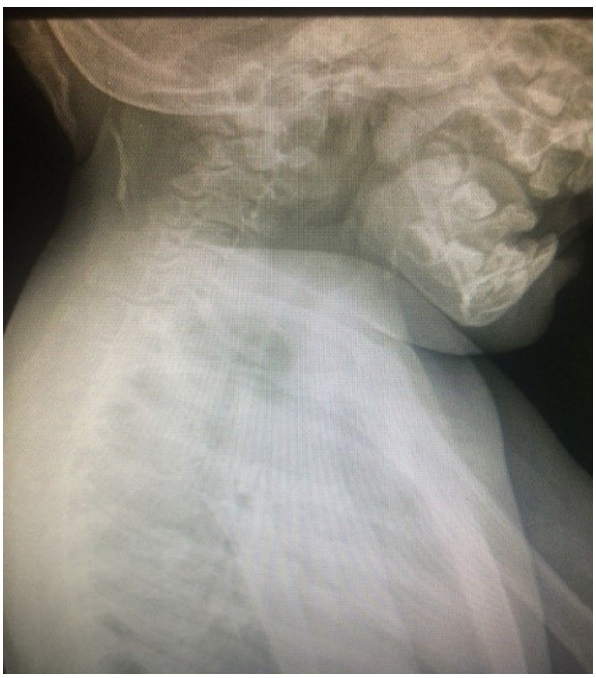

Fig. 9. X-ray examination of Patients 3 showing ossifications in neck area

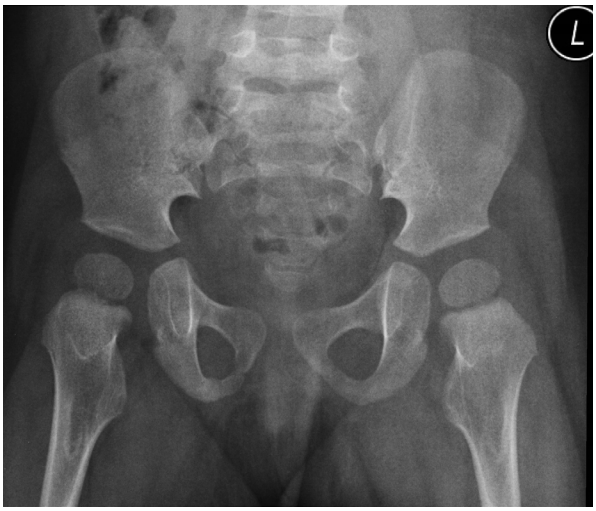

Fig. 10. X-ray examination of Patients 3 showing calcium-saturated bands in the soft tissues of the upper part of the lumbar spine, the area of the buttocks and the sacrococcygeal area and deformities of the necks of both femur and the head of the left femur

Discussion

The above-described cases allow us to conclude that the correct diagnosis at the stage of oedema, before additional ossifications are formed, is associated with several difficulties and the time period between the occurrence of the first symptoms and the establishment of the diagnosis ranged between 3 months and 2.5 years, the latter being the case with an atypical course. New bony tissue was formed outside the skeleton, mostly in the muscles, ligaments, fascia and tendons, avoiding the muscles of the diaphragm, tongue, heart, eye and smooth tissues, which agrees with published reports.[9] There were erroneous suspicions of cancer, including sarcoma, osteosarcoma, lymphomas and desmoid tumours. In consequence, the decision to perform surgery was made, after which usually the ossification process was intensified, which was observed in two of the described cases.[12] Positive support for the diagnostic process was the presence of typical phenotype defects, observed immediately after birth, the presence of which was found in each of the described cases. These defects comprise microdactylia and valgus of the big toes occurring as a result of the deformity of the first metatarsal bone and lack of formation of the interphalangeal joint, and, sometimes also clinodactyly of the fifth toe.[13-16] In two children, a shortening of the thumbs was observed, yet this defect occurred less frequently. Additionally, in all the described cases, an X-ray revealed deformities of the vertebral bodies in the cervical spine, together with a bony adhesion of C2-C7, short and wide femur necks and chondorosteophytes within the proximal tibia, which is a feature frequently reported in many publications.[14,16-19]

In spite of the presence of the typical p.R206h homozygotic mutation in the sequence coding of the ACVR1 gene, disease development may be varied. A typical picture is characterized with periods of exacerbations, within which new bone is usually formed. Also, the reports declare that the flashes are mainly triggered by traumas, surgeries, biopsies, intramuscular injections, muscle overloads and infections, but can also be spontaneous.[20,21]

In two of the children described above, progressive heterotrophic ossification had a specific course, involving first the areas of the nape, neck, spine area, back and shoulder girdle, extending then to the distal segments of the upper extremities, chest, and through the abdominal integuments to the lower limbs. Initially, the lesions formed nodular subcutaneous infiltrations, above which the skin was unchanged and sometimes with vessels visible through the skin. Gradually, these lesions hardened forming a new bone located in the muscles and around the joints and were followed by limitation of mobility, stiffness and sometimes, subluxations of the joints.[19,22,23] Incidentally, differences in the ossification sequence are observed in spite of the presence of a typical mutation , as seen in Case 2. Each of the described cases, presented different dynamics in symptoms development and involvement of the motor organ: beginning with a very aggressive course impairing physical activity in a very short period of time (Case 3) ending with a latent form, without flashes even at large traumas, the onset of which had an atypical location (Case 2).[24] The results of laboratory tests were normal, apart from an elevation of alkaline phosphatase activity in the periods of exacerbation in Cases 1 and 3.[25] The diagnosis may be facilitated by conventional X-rays in which the foci of heterotopic ossification may be visualised. Such examinations as CT or MRI are pointless in the early stage of the disease. For a correct diagnosis, it is enough to diagnose typical big toe anomalies and to confirm the mutation of the *ACVR1* gene.[26,27]

Currently there is no efficient therapy of FOP, which could prevent the formation of new ossifications. Attempts to the resect a new bony tissue result in an even larger and more intensive ossification in the locations of an intervention, which happened in Case 2. The guidelines for a symptomatic treatment are presented on the IFOPA website. The application of GCs in the periods of exacerbations seem to be the most effective in treatment. In the case of the involvement of large joints, the head and the neck, mega-doses of GCs, administered parenterally are recommended. This treatment might lead to the fast regression of oedematous lesions and alleviate strong pain while at the same time improve joint mobility – as was observed in 2 cases. As a standard supportive therapy, non-steroid anti-inflammatory drugs, leukotriene inhibitors and agents stabilizing mast cells are used, yet they do not affect disease activity. Attempts of long-term immunosuppression, according to

the published data, render positive results in some cases, yet the general use of drugs from this group is not recommended. Some significant improvement was made after the application of bisphosphonates, which were administered in 2 of the 3 described cases, obtaining a decrease in the number of flashes and the reduction of the applied doses of GCs.[12,28,29] Some hopes have been placed in a procedure of allogenic bone marrow transplantation, which lead to the elimination of lymphocytes which are genetically programmed to produce an excess of BMP-4. The studies performed on an animal model revealed that an allogenic bone marrow transplantation followed by immunosuppressive therapy reduced extra-skeletal ossification, although it did not result in remission of the disease.[8] High hopes have also been placed in the use of palovarotene, the efficiency of which is currently being evaluated in clinical studies.[30]

Conclusion

Until recently, there has been no efficient therapy which could slow down the natural course of the disease and currently the disease is treated as incurable. Of key importance from the perspective of patients is early diagnosis and, more importantly, preventing traumas, surgical procedures, intramuscular injections, sparing dental treatment and ensuring avoidance of airway tract infections.

Acknowledgments

The authors thank prof. Frederick Kaplan (The Isaac & Rose Nassau Professor of Orthopaedic Molecular Medicine and Chief of the Division of Molecular Orthopaedic Medicine at the University of Pennsylvania School of Medicine) for inspirations, directions and discussion during writing this article. The authors thank prof. David Aebisher for his help in text editing and English corrections.

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European Journal of Clinical and Experimental Medicine
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