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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

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ORIGINAL PAPER

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Time estimation and time perceiving in patients receiving intravenous anaesthesia for endoscopic procedures

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ABSTRACT

Introduction. The basic goals of the study were to analyze how patients receiving intravenous anaesthesia for endoscopy produce one-minute time intervals after anaesthesia, and to characterize the relationship between attitude towards time and the production of one-minute intervals.
Material and methods. Twenty four intravenously anesthetized patients constituted the Anaesthesia Group and 25 nonanesthetized patients formed the Control Group. The Mini-Mental State Examination and the Sense of Coherence Meaningfulness Subscale were used to recognize the problem of dementia and depression, the Time Metaphors Questionnaire was used for the assessment attitudes toward time. Time production of one-minute was measured three times in each of four sessions.
Results. The tested participants of both groups shortened the one-minute intervals. Duration of anaesthesia did not affect the time production. Perceiving time as empty and meaningless was related with misestimating time after colonoscopy.
Conclusions. Time interpretation by using metaphors and time production are related with each other.

Keywords: time perception, time metaphors, anaesthesia, endoscopy.

Introduction

Emotions and cognition are tightly bound entities that have clear functional and organic basis [1]. Both of them can be disturbed in hospitalized patient. Negative emotions, like fear and anxiety influence the patients undergoing anaesthesia regardless of age, and this impact has been extensively discussed in the literature [2–4]. On the other hand, cognition is affected by perioperative stress and anaesthesia influencing the quality of life which has also been well described by Steinmetz et al. [5]. Emotions and cognition are also engaged in the process of time production [6]. We know from the previous studies that anxiety and depression affect the intestinal motor function [7, 8]. These facts inspired us to construct the study assessing the relationship between emotional and cognitive aspect of time perception in relation to intravenous anaesthesia for endoscopic procedures. Especially that brain regions responsible for the interval timing (corticostriatal circuits and dopamine neurons) are closely related to cognitive phenomena and can be discussed as a part of human cognitive func-

tioning (working memory, motor functions, decision making, etc.) [9].

The aim of the study was to analyze the impact of receiving intravenous anaesthesia for endoscopic procedures on producing one-minute intervals of time, and to assess the connection between perceiving time before anaesthesia and the production of one-minute interval after colonoscopy.

Our study poses the following main research questions: 1) What influence do anaesthetics administered during colonoscopy exert on the generation of the one-minute time interval?; 2) Does the manner patients perceive time prior to colonoscopy have any relation to generating one-minute time interval following the procedure?

Colonoscopy is a highly invasive, stress-inducing diagnostic procedure. It is a procedure which in a controlled and reversible way results in sleep, retrograde amnesia, analgesia and the inhibition of sympathetic reflexes. The substances used for this kind of anaesthesia impair psychophysical functions for a few hours after their administration [10]. We suggested the hypothesis that patients after anaesthesia could develop alterations in perception of time when compared to nonanaesthetized controls.

The patient's attitude to diagnostic and treatment procedures, as well as their approach towards the time spent in hospital and the relations with medical personnel, are of utmost importance in therapy. Negative emotional state, especially severe anxiety, may considerably hinder the treatment, to the extent that it can even render certain treatment or diagnostic procedures to be impossible to perform [7]. Since one's attitude to time is closely related to one's emotional state [11–16], and to approach to the treatment [17, 18] it may be assumed that the perceiving of time shall be closely linked to the manner in which the patient's psyche deals with experiencing a serious diagnostic procedure and the recovery following it. Hence, we assume that patients who evaluate time in a negative way will more considerably misestimate time after a diagnostic procedure under anaesthesia.

Material and methods

This study was performed with permission from the local bioethical committee (Bioethical Committee of Poznan Medical University, Permission No. 427/10, May 6, 2010). Patients gave written consent for participation in the study.

The following psychological tests were used during the study.

The Time Production Method of time duration judgement was used [19]. The estimation of time intervals is interpreted psychologically through the functioning of the inner clock the human being is equipped with. Additionally, estimating the duration is perceived as a cognitive construct under the influence of attention and memory [20]. From the neuropsychological perspective it should be stressed that there are a number of mechanisms involved in the process of time estimation, as well as various regions of the human brain: the cerebellum ('automatic system'), the prefrontal cortex, the lower part of the parietal lobes ('cognitive system') and the basal ganglia (mainly striatum), the thalamus and the anterior cingulate cortex. These structures are related to other cognitive functions, such as attention, memory, visual-spatial coordination or language skills. The presented systems operate simultaneously [9], their functioning may be disturbed by, among others, the effect of psychoactive substances (drugs or medicines) [21–23].

Time production of one-minute was measured by means of a stopwatch three times in each of the sessions: initial, 1.5, 3, and 6 hours after anaesthesia. The participants of the study were asked to produce one-minute intervals three times. The examiner said 'start' to the participant and the tested patient was asked to say 'stop' once one minute of time had passed. The participant was not instructed whether or not to count in silence.

The perceiving of time was assessed with the Time Metaphors Questionnaire (TMQ) [24]. This questionnaire is composed of ten scales. The Constructive Time (CT) scale (alpha =.93, 20 items) contains items which describe time as directed towards the truth and the achievement of goals (for example, 'Time is a way to put plans in operation'). The Friendly Time (FT) scale (alpha =.86, 12 items) characterizes time as a friend and a teacher (for example, 'Ever-ageing time teaches all things'). The Awareness of Mortality (AM) scale (alpha =.84, 9 items) includes metaphors of time as finite (for example, 'Time is an incurable disease'). The Hostile Time (HT) scale (alpha =.86, 10 items) describes time as a disappointment and an enemy (for example, 'Time is a disappointment'). The Confusion in Time (CO) scale (alpha =.82, 9 items) characterizes time in terms of a power which is out of control (for example, 'Time flows beside me'). The Rapid Passage of Time (RP) scale (alpha =.85, 10 items) includes metaphors representing time as a speedy vehicle (for example, 'Time flies like an arrow'). The Significance of the Moment (SM) scale (alpha =.77, 7 items) includes metaphors indicating the

necessity of making the most of each moment of life (for example, 'Each moment is worth one's weight in gold'). The Chaotic Time (CH) scale (alpha =.80, seven items) consists of items describing time as turbulent (for example, 'Time is like a stormy ocean'). The Subtle Time (ST) scale (alpha =.79, six items) characterizes time as peaceful (for example, 'Time is like incense smoke'). The Empty Time (ET) scale (alpha =.67, five items) describes time as boredom (for example, 'Time is like a drowsy afternoon').

The participants have to indicate 'how they imagine time' [24] on a four-point scale ranging from 'very appropriate' to 'very inappropriate'. Results of the research indicated that people high in Extraversion, Openness, Agreeableness, Conscientiousness and low in Neuroticism (measured by NEO-FFI by Costa, McCrae) generally evaluated time positively (high score on Constructive Time, Friendly Time, Significance of the Moment, Subtle Time). In turn participants with high score on Neuroticism scale perceive time usually as speed, wild and chaotic [12]. Friendly Time scale correlate positively with realization of aims. Negative time evaluation was linked with time pressure, fatalism and hedonism measured by Time Perspective Questionnaire AION-2000, Nosal & Bajcar [12]. Relation between TMQ and PANAS (The Positive and Negative Affect Schedule, Watson, Clark and Tellegen) indicated that perceiving time as friendly and constructive were linked with positive emotions, and conceiving time as enemy were connected with negative emotions. There were no significance correlations between positive evaluation of time and negative emotions [24]. Time Constructive and Time Friendly scales correlated positively with a quality of life measured by CASP-19 by Higgs and others [25] and with the purpose in life measured by Noo-Dynamics by Popielski [26].

The Mini-Mental State Examination (MMSE) [27] was used to recognize the problem of dementia, which was the major exclusion criterion. This method is a well-known test used for the screening of cognition in depressed patients and for diagnosing cognitive dysfunction, mainly towards dementia. The Polish MMSE version normalized by Stańczak [28] was used in this study (alpha =.88; 30 items).

The Meaningfulness Subscale (MS) from the Sense of Coherence Scale (M-SOC) [29] was used as a method for recognizing the problem of depression – the second major exclusion criterion [30, 31]. This subscale is composed of eight items. It measures the level of feeling towards important fields of life worth emotional involvement and energy expenditure. The answers to the statements are scored on a 7-point scale. The Polish M-SOC version was used in this study (alpha =.71) [32].

All tests were performed in standardized conditions (air-conditioned quiet room with constant artificial light, similar time of a day).

Between June and September 2010, 40 patients hospitalized in the clinics of the Poznan University of Medical Sciences, were enrolled in the study: 24 (16 women and 8 men) who underwent a colonoscopy under intravenous anaesthesia constituted the Anaesthesia Group and 25 patients (19 women and 6 men) who did not receive such treatments formed the Control Group. To exclude those with pre-existing cognitive disturbances and depression, a screen was performed using the Mini-Mental State Examination (MMSE≥24 pts) and Sense of Coherence Meaningfulness Subscale (M-SOC = 34 pts) [30, 31, 33]. Patients in Anaesthesia Group and Control Group were asked to participate in the study and give their informed consent. Patients were clinically examined by a physician with regard to dehydration, ion imbalances (potassium and sodium), severe anaemia (Hb = 10 mg/dL), and thyroid dysfunction; the presence of any of these pathologies was a criterion for exclusion. The Anaesthesia Group patients were premedicated with oral midazolam 0.1–0.15 mg/kg and transferred to the operating room. If the patients were anxious before the procedure, additional sedation was induced with midazolam 1–2 mg iv (Midazolam, WZF Polfa). Patients were anesthetized using intravenous propofol (Plofed 1%, WZF Polfa) 1–2 mg/kg was used. Analgesic doses of fentanil (Fentanyl, WZF Polfa) 1–2 μg/kg iv were used. During the anaesthesia, vital signs were monitored, and 1000 ml Sterofundin (Braun) was infused continuously to prevent dehydration. Vital signs were recorded and no adverse events were observed.

Statistical analysis

Statistical analysis was performed using PASW Statistics v.19 (2011) for Windows. The demographic data, results on TMQ, time production and the duration of anaesthesia were presented as mean with standard deviation (SD). The differences in demographic data between the participants of the groups, time production test, TMQ and the relationship between the duration of anaesthesia and the production of one-minute intervals were tested by t-test and p = 0.05 was considered statistically significant. The results on the time production test were analyzed in the parallel sessions

between the groups as well as between the consecutive sessions within the groups. Correlation between TMQ and Time Production results were analyzed by Pearson's coefficient and p = 0.05 was considered statistically significant.

Results

Demographic characteristics

The demographic characteristics are presented in **Table 1**.

The groups did not differ in terms of the demographic characteristics. General level of scholar education in studied participants has been various: ten (AG: 7, CG: 3) received primary education, 25 (AG: 11, CG: 14) received secondary education and 14 subjects (AG: 11, CG: 3) received higher education.

The patients did not present pre-existing cognitive disturbances when screened with MMSE. The results on M-SOC did not differ between the groups and were above 40 points, which is adequate to a range for mean population (t-test; p = 0.05) [31].

Time production of one-minute interval in three trials

Table 2 presents mean results of generating one-minute time intervals in the Anaesthesia Group and the Control Group, as well the differences between them measured by means of the t-test. In order to analyse the degrees of misestimation of one-minute time intervals, the results (that is, the numbers of seconds estimated by the subjects to represent one minute) were converted into a time interval misestimation ratio defined as the absolute difference between 60 seconds and the number of seconds reported by the subjects. All mean 1–minute intervals produced by the participants of both groups were shorter than the real duration. There were no significant differences between the time intervals generated by the groups of hospitalized patients. There were also no significant differences in the time misestimations between the Anaesthesia Group and the Control Group. There were, however, significant differences in the time misestimations between the group of patients who received midazolam immediately prior to colonoscopy (N = 11) and the group of patients who did not receive this substance (N = 13). Significant differences (p < .05) occurred in two trials prior to the procedure and in two trials 1.5 hour following it. The patients who had been administered midazolam were less accurate in generating the one-minute interval in comparison with the patients who had not received it. The generation of one-minute intervals did not differ between the groups in the trials 3 and 6 hours after the procedure.

The influence of the anaesthesia duration on the production of one-minute intervals

The duration of anaesthesia was 10–60 min. (mean 36.74 min; SD 12.76). By t-test, we analyzed the relationship between the results on the production of the one-minute intervals and the duration of anaesthesia. We observed no statistically significant relationships (p = 0.05). There were no significant connection between the degrees of misestimation on the length of anaesthesia.

TMQ scales relating to one-minute time production

The correlation coefficient between the degree of misestimations in the generation of time intervals and the conception of time in metaphors was calculated. In the Anaesthesia Group there was one significant correlation (r=.47, p<.05) between the Empty Time scale results and the degree of misestimations in the gen-

Table 1. Characteristics of the study group (age, height, BMI)

	Anaesthesia Group N = 24, 16 women, 8 men	Control Group N = 25, 19 women, 6 men
Minimum age	20	19
Maximum age	77	75
Mean age (M)	42.46	42.96
Age-standard deviation (SD)	16.83	17.18
Minimum height	145	152
Maximum height	182	195
Mean height (M)	165.54	166.64
Height-standard deviation (SD)	10.25	10.37
Minimum BMI	17	17
Maximum BMI	38	35
Mean BMI (M)	23.17	23.13
BMI-standard deviation (SD)	5.49	5.48

Table 2. Mean scores (M), standard deviations (SD) of the results of the Time Production Method (TP) and the degrees of time misestimation (TPZ), as well as differences between them (t-test) in the AG and the CG

	AG (N=24)		CG (N=25)		
	M	SD	M	SD	t
TP I 0	49.83	21.38	43.83	18.17	6
TP II 0	52.35	20.92	47.14	18.17	5.21
TP III0	52.25	20.44	47.14	16.81	5.11
TP I 1.5	44.28	21.35	40.51	18.07	.24
TP II 1.5	47.25	20.26	43.07	18.5	.42
TP III 1.5	49.55	21.51	44.98	19.56	.65
TP I 3	47.09	17.26	45.30	13.62	1.79
TP II 3	51.21	18.76	50.11	17.17	1.1
TP III 3	51.45	19.22	51.23	16.70	.22
TP I 6	47.38	15.40	48.98	16.26	-1.61
TP II 6	49.14	14.40	50.74	17.39	-1.60
TP III 6	50.59	14.93	51.42	18.38	-.82
TP Z I 0	19.94	12.24	19.92	13.75	.01
TP Z II 0	18.18	12.41	17.39	13.7	.21
TP Z III 0	19.12	10.98	16.14	13.55	.83
TP Z I 1.5	21.43	15.28	17.08	12.46	1.05
TP Z II 1.5	19.89	12.91	15.02	11.65	1.34
TP Z III 1.5	20.22	12.22	14.4	11.38	1.67
TP Z I 3	16.96	13.11	15.06	13.21	.51
TP Z II 3	16.31	12.44	13.97	13.91	.61
TP Z III 3	16.64	12.51	12.39	14.11	1.11
TP Z I 6	16.04	11.62	14.19	13.45	.51
TP Z II 6	14.72	10.22	14.43	13.22	.08
TP Z III 6	14.23	10.19	13.55	14.32	.18

TP I, TP II, TP III – time estimation: trial 1, trial 2, trial 3, respectively
TP Z I, TP Z II, TP Z III – time misestimation in trial 1,2 and 3, respectively
0, 1.5, 3, 6 – estimation of time: initial, 1.5, 3 and 6 hours following the procedure, respectively

eration of time intervals in the first trial 1.5 hour following colonoscopy – the more frequently the studied patients perceived time as ennui and emptiness, the larger the discrepancy between the generated interval and the target one-minute were. Interestingly, there was no significant relation between the perception of time as emptiness and the generation of time intervals on the day preceding colonoscopy.

Discussion

The primary purpose of this manuscript was to look into the effect on generating time intervals by the patients who were administered general anaesthetics during colonoscopy and to analyze the relationship between perceiving time and the production of one-minute intervals. The postulated hypotheses were partly verified. The first hypothesis concentrated on the impact of general anaesthetics on time interval estimation. The research showed that, contrary to what had been supposed, such substances do not exert any greater influence on the perception of time on the psychophysical level and, what is more, the duration of anaesthesia is also irrelevant. The quick review of the basic results on time production is an optimistic piece of information. Riphaus et al. [34] conducted a study on the influence of short sedation for endoscopic procedures with the application of propofol or midazolam/pethidine. They tested patients 2 hours after anaesthesia and found no differences when tested with the Number Connection Test and a driving simulator. Sanou et al. found that higher cognitive functions (memory, attention, language comprehension and planning) were impaired up to 3 hours after propofol anaesthesia, but recovered 6 hours after it [35]. Interestingly, even elderly patients tested by Kubitz et al. recovered to the baseline results in simple reaction times, critical fusion frequency and short-term memory 2 hours after propofol/remifentanyl anaesthesia, thus proving the usefulness of short-acting anaesthetics in the practice [36].

The second hypothesis, referring to greater dysfunctions in time conception among patients perceiving time as a negative phenomenon prior to colonoscopy, was partly supported. The patients who often

associate time with boredom, emptiness and nearly pointless existence found it more difficult to generate accurate time intervals, as compared with the patients who seldom thought of time in such terms. We might draw a conclusion that, even if time is seen negatively, but one sees some sense in it and fills it with time-consuming activities, it is possible to regain balance faster after unpleasant experiences. It should be mentioned that high results in the Empty Time scale strongly correlate negatively with the feeling of a sense in life [12, 24]. There were also interesting differences between the patients experiencing strong anxiety before colonoscopy (i.e. those who were additionally sedated with midazolam) and the patients who were not administered this drug. The patients, who received midazolam, more frequently misestimated time prior to colonoscopy and in the first test after it. Thus, one can say that anxiety exerts a strong influence on the inner clock. Large degrees of misestimation occurred after colonoscopy, solely in the first trial, hence the influence may not have been exerted by the additional sedative, but by the negative emotional state. One can consider the possible residual influence of midazolam on the postoperative performance, although midazolam has a short half-life and small doses used to achieve the similar level of sedation according to Ramsay scale should not affect the Time Production test some hours later.

It is worth to note also that the tested participants of both groups shortened the one-minute intervals. We know from the literature that emotions and cognition are tightly-related items [1]. If we consider the relationship of emotions and perception of time, we know that positive emotion induces impatience and time underestimation (shorter than real). Negative emotion triggers anxiety, gives the experience of time contraction and overestimates time (longer than real) [37, 38]. In the light of the presented data, the participants could have had more positive feelings during hospitalization, because they tolerate the short stay on the ward well. It is probable that the possible therapeutic effect attributed to the colonoscopy was important for emotional state of the patients. One may also say that the very diagnostic process alone (and not the diagnosis nor treatment) relieves suffering and uncertainty – communication as a process, but not the final message - as a result of communication. It is also worth remembering that the period of awaiting to be admitted to a clinical ward for diagnosis is usually long in Poland, hence the very admittance into a clinical ward is therapeutically significant. The patient's state can improve just as a result of seeing the end of uncertainty and anticipation which relieves from suffering.

As regards limitations in our study, first and foremost the small population of subjects and an imbalance between the number of participants of each of the sexes. This situation resulted from the considerable reluctance on the patients' side and the definite majority of women among the patients hospitalized on the gastroenterology ward.

Conclusions

The results show that there were no relations between duration of anaesthesia and the time production. The conception of time as boring and meaningless was linked with larger misestimations of time intervals following colonoscopy. Anaesthesia is an important part of perioperative medicine and there is a growing interest in using anaesthetics in other fields been constantly spreading. The meaning of time perception may become one of its expressions.

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REVIEW PAPER

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Effectiveness of regular physical activity on exercise tolerance and biochemical parameters in high-risk prostate cancer patients during radiotherapy

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ABSTRACT

Introduction. Radiotherapy (RT) is an important modality for curative treatment in high-risk prostate cancer (PCa) patients that improves overall survival, however, it may cause unfavorable changes in physical fitness, increased risk factors for cardiovascular and metabolic complications. The effects of physical exercise on aerobic capacity and on serum levels of liver and renal function indices in PCa patients undergoing RT are still unknown.
Aim. The purpose was to assess the impact of supervised physical activity on aerobic capacity, hemorheology and metabolic biomarker levels in high-risk PCa patients during RT.
Material and methods. Seventy-two men were randomly allocated to two groups before RT for high-risk prostate carcinoma. Thirty-six men conducted physical exercise (EG) and the other 36 men were a control group (UC). Outcomes measured were 6-Minute Walk Test (6MWT), blood parameters, serum levels of hepatic and renal function biomarkers (urea, creatinine, alanine and aspartate aminotransferase, PSA) in a modified shuttle test before and after RT.
Results. After RT, decreased diastolic blood pressure (before test $p = 0.05$) was observed in the EG and in the UG, there was an increase in resting heart rate ($p = 0.017$), a decrease in walking distance ($p = 0.036$), and an increase in Borg fatigue score during the 6MWT. There was no statistically significant change in renal biomarkers or PSA in the liver in either group.
Conclusions. Physical activity in prostate carcinoma patients during RT improves capacity tolerance with a decrease in Borg fatigue score, but this activity did not influence on serology outcomes or other blood indicators during RT.

Keywords: exercises, radiation therapy, oncology, supportive care, rehabilitation.

Introduction

Radiotherapy (RT) is an important modality for curative treatment in high-risk prostate carcinoma (PCa) patients that improves disease-free and overall survival, especially in locally advanced or higher-risk disease [1]. However, in this group of patients, it may cause unfavorable changes in quality of life, and physical fitness, as well as increases in stress or fatigue [2–5]. Patients with PCa have a high level of interest in life-style changes and are a receptive population for exercise intervention, which, if continued after RT, may have long-term general health benefits in this population of older men [6] Additionally, androgen deprivation therapy (ADT) leads to a number of adverse effects, including deterioration of the musculoskeletal system and increased risk factors for cardiovascular and metabolic complications (e.g. negative lipoprotein profile, abdominal obesity) [7].

On the other hand, the important implications of stress generating situations (such as cancer) to changes in blood lipid levels have been widely documented [8]. Apart from its relationship with blood lipid levels, cardiovascular risk has also been related to specific blood parameters [9,10]. Increased values of these biochemical parameters are typically associated with liver pathologies. Similarly, urea and creatinine levels are generally considered to be indicators of renal function, although these measures may also be altered in liver and muscle diseases, respectively [11].

The beneficial effect of physical activity on general health and well-being and on mood and mental well-being is well documented and accepted. Exercise training can induce changes in serum concentrations of numerous laboratory parameters [11]. Physical activity can improve cardiovascular efficiency, increase cardiac output and stroke volume, decrease resting heart rate, lower exercise heart rate, and improve ventilation and transport of oxygen from the environment to the cells. Vera and co-authors [12] demonstrate that daily exercise training for a period of one month induces noteworthy modifications of diverse blood biochemical parameters. Exercise training is associated with improved hemorheology and can increase blood volume through an increase in plasma volume and red blood cell mass [13].

It is well documented that physical exercise in patients with localized PCa undergoing RT improved cardiac fitness, flexibility, muscle strength, and overall quality of life, as well as prevented fatigue [2, 3, 5]. The effects of physical exercise on aerobic capacity and on serum levels of liver and renal function in PCa patients undergoing RT are still unknown. Therefore, the aim of this study was an observation of the impact of regular physical exercise on selected blood parameters and endurance tolerance in high-risk PCa patients undergoing RT.

Material and methods

Setting and Subjects

This study was conducted between January 2013 and June 2014. The Bioethical Committee at the University of Medical Sciences approved all study procedures (No 10/12). Detailed written and verbal information was provided to participants concerning assessment and training protocols. Participants were informed of the voluntary and confidential nature of the study and were free to withdraw from the study at any time. Informed consent was obtained before participation in the study.

Patients participating in the study met the following eligibility criteria based on a diagnosis of histologically confirmed prostate adenocarcinoma. The definitive RT group had high-risk PCa (T3 or Gleason score > 7 or PSA >20 ng/mL) and a maximum prostate gland volume of 80 cc, age of patients between 18 and 75 years old, scheduled androgen deprivation therapy (ADT) (LHRH analogue every 3 months) planned to continue for a total period of 36 months (3 to 5 months prior to RT, during and after completion), no distant metastases, no endocrinological, rheumatic, or cardiac diseases resulting in circulation failure (above Stage II Heart Failure according to the New York Heart Association), no absorption disorders, no other tumors, and good general health (ECOG performance status 0–1).

Exclusion criteria were distant metastases and/or disease progression resulting in RT or the introduction of chemotherapy, insufficiently controlled arterial hypertension, insufficiently controlled metabolic diseases, withdraw from the study before the 3-month period, or death of a patient during the course of the study. For the analysis of the relationship between heart rate (HR) and 6-min walk distance (6MWD), we excluded any subject on medications that could affect HR, such as β-blockers or calcium channel blockers. Eligible participants were identified by their oncologist.

Design

This study was a two-arm pilot randomized controlled clinical trial. All patients underwent a series of baseline assessments over 2 days, including completion of an on-study form, clinical record form and a 6-minute walk test (6MWT). After completing all baseline assessments, patients were stratified by diagnosis (PCa) and, subsequently, randomized, using a randomization to the control condition consisting of radiotherapy alone or the intervention condition consisting of radiation therapy plus an individually tailored exercise program. Patients randomized to the control group were instructed not to begin any new formal physical exercise programs. This study was not fully blinded; however, the condition allocation was concealed from the patient and physiatrist until after the completion of the baseline assessments. A clinical research coordinator obtained patient consent, collected all the self-reported assessments, and explained the exercise program to participants. The study statistician and data managers remained blinded at all times.

Radiation treatment

All RT patients received a total dose of 76 Gy in 38 fractions. Prior RT neoadjuvant ADT was started, and then given during the course of RT and thereafter up to 24 months. In the first phase of therapy the pelvic

lymph nodes with prostate gland and seminal vesicles were included to the dose of 46 Gy at 2 Gy fractions. In the second phase of therapy the irradiated volume was limited to prostate gland plus seminal vesicles to a total dose of 76 Gy. Quality assurance for RT was achieved by daily fiducial marker matching. The clinical target volume included the prostate gland and seminal vesicles but not the pelvic lymph nodes. Patients were positioned in the supine position on the therapeutic machine with a comfortably full bladder and empty rectum [1, 14]. The time interval between beginning neoadjuvant ADT and RT was more than three months but not longer than five months. For each patient, the following organs at risk with constraint doses were established: rectum, bladder, and femoral heads.

Assessment

In the classified group of patients, the following schedule was used: Assessment I (Baseline) one week before the onset of RT and ADT and Assessment II, 1 week after the end of RT (after 8 weeks of regular physical exercises).

6 – Minute Walk Test

Aerobic capacity was estimated using a 6 MWT protocol, which has been used extensively in clinical exercise trials to estimate aerobic capacity in cancer patients [15, 16]. Recent studies concluded that this method possesses excellent measurement properties, is better tolerated, and is more reflective of activities of daily living than any other walk test in use [15, 17].

Participants were given a short warm-up and then asked to walk for a total of 6 minutes, covering as much distance as possible. Two 6MWTs were performed following ATS guidelines [18]. The evaluated parameters were 6MWD in m and changes in oxygen saturation (SpO2) and HR during exercise measured via pulse oximetry with a VM-2101-Finger Oximeter – Viamed, United Kingdom. The longest 6MWD of two tests (performed the same day and separated by 20 min) was the primary outcome measure. The 6MWT was followed by a short cool-down period and conducted in a hospital corridor, 30 m long. Patients were instructed to walk the corridor from one end to the other as many times as possible within the permitted time. Upon completion of the test, the total distance walked was recorded and used to estimate aerobic capacity. Secondary measures included fatigue (dyspnea) after test using a modified Borg scale (0–10) [18].

Blood parameters analysis

Venous blood samples were taken to measure prostate-specific antigen (PSA), hemoglobin (Hb), white blood cells, red blood cell neutrophils, lymphocytes, monocytes, platelets, serum lipids (total cholesterol, high- and low-density lipoprotein cholesterol – HDL and LDL), and triglycerides – TG). Serum parameters (urea, creatinine, alanine aminotransferase – ALT and aspartate aminotransferase – AST) were also obtained and processed by a centralized laboratory. Biochemical markers were measured using the Cobas 6000TM clinical chemistry analyzer (Roche, Mannheim, Germany). Hematological indices (complete blood count, hemoglobin) were analyzed in EDTA-blood with the XT-2000i TM (Sysmex Corporation, Kobe, Japan).

Physical exercise

The intervention group conducted physical exercise 5 days per week. The study exercise program began before RT with aerobic exercises to evaluate the effects of RT and ADT prior to the initiation of exercise. The aerobic activities were completed either alone or in groups and took place at the Rehabilitation Ward in the Greater Poland Cancer Center under the supervision of at least one physiotherapist. The optional exercises included brisk walking, running outside or on a treadmill, and various cycling activities. All activities lasted approximately 50–55 min. The workout consisted of a 5-min warm-up and 40 min of one of the activities, followed by a 10-min relaxation period. The physical activity was moderate, with a maximal heart rate of 65–70% of the maximum heart rate (220 – age) according to American Cancer Society recommendations [19].

Study organizers verified patients' exercise programs through physical activity notebooks that were checked by a physician in the rehabilitation department once a week.

The control group performed their daily physical activity on their own. Patients in this group were given general recommendations for daily physical exercise [20].

Exercise program adherence

In the course of the study, some breaks from the exercises were allowed, which were to last no more than two days per 8 weeks. Study organizers verified patients' exercise program through physical activity notebooks that were checked by a physician in the rehabilitation department once a week.

Diet

PCa patients in our study provided a normal, balanced diet (not restricted), which we observed using Mini Nutritional Assessment (MNA) [21].

Statistical data

The statistical data was analyzed using STATISTICA software (version 10.0 StatSoft, Poland). The results of anthropometric and 6MWT measurements, as well as blood parameters were analyzed. The quantitative data was described through mean and standard deviation. The Shapiro-Wilk test was used to assess normal distribution compatibility. The differences between the results were described using a two-sided Student's t-test and Wilcoxon's test for connected variables, taking into account the size of the 95% confidence interval (CI). The results with $p \leqslant 0.05$ were regarded as statistically significant.

Results

Participants

Seventy-three patients were randomly allocated into two groups: 36 men were allocated to the aerobic exercise training group (EG) and the other 37 men comprised the usual care group (UG; one of them was excluded from the study due to his absence during Assessment II). There were no significant differences between the groups at baseline in age or BMI. Demographic data of the participants are summarized in **Table 1**.

Changes in Objectively Measured Outcomes

The results from the paired t-tests in **Table 2** demonstrated differences of 6MWT parameters between the treated patients. In the exercise group (EG), there was a statistically significant increase in oxygen saturation before the 6MWT and a decrease in diastolic blood pressure (before test – p = 0.05)

Table 1. Baseline characteristics of the study groups.

Characteristic	Overall Sample (n=72)	Exercise Training Group (n = 36)	Usual Care Group (n =36)	p
Age [years] (Mean+/-SD)	66.23+/-4.94	65.7+/- 6.2	67.9 +/- 4.9	0.161
Weight [kg] (Mean+/-SD)	83.25+/-7.50	83.12+/- 8.8	85.43+/- 6.7	0.882
BMI [kg/m2] (Mean+/-SD)	28.69+/-3.4	26.42+/-2.8	29.25+/-3.7	0.386
PSA [ng/mL] (Mean+/-SD)	4.23+/-2.26	4.08+/-1.57	4.73+/-2.28	0.226
Gleason score(Mean+/-SD)	6.76+/-1.89	7.02+/-1.20	6.88+/-1.92	0.386

SD, standard deviation; BMI, body mass index; PSA, prostate specific antigen

Table 2. Effects of Physical Exercise on 6MWT Outcome Parameters in Prostate Cancer Patients Receiving Radiotherapy

Characteristic			Baseline		After treatment		Change From Baseline to After Treatment		
			M	SD	M	SD	M	95% CI	p
SpO_2 [%]	before 6MWT	EG	97.41	1.05	97.96	0.86	+0.55	-0.95 to -0.15	0.008*
		UC	97.56	1.34	97.17	1.15	-0.39	-0.03 to +0.81	0.070
	after 6MWT	EG	97.96	1.08	97.89	1.04	-0.06	-0.41 to +0.55	0.773
		UC	97.30	1.60	97.26	1.83	-0.04	-0.61 to +0.70	0.892
Systolic BP [mmHg]	before 6MWT	EG	143.13	17.77	138.27	16.10	+4.86	-1.11 to +10.83	0.106
		UC	144.26	15.57	143.26	13.87	-1.00	-5.28 to +7.28	0.744
	after 6MWT	EG	148.82	18.89	146.03	17.14	-2.79	-4.30 to +9.89	0.427
		UC	145.21	19.37	148.47	20.44	-3.26	-9.26 to 2.73	0.271
Diastolic BP [mmHg]	before 6MWT	EG	86.13	8.32	82.00	12.07	-4.13	0.45 to 7.81	0.028*
		UC	80.52	12.56	83.91	10.07	-3.39	-9.15 to +2.37	0.235
	after 6MWT	EG	85.72	10.23	83.86	9.31	-1.86	-2.47 to +6.20	0.386
		UC	78.95	11.25	83.78	13.71	+4.82	-9.64 to -0.00	0.049*
Pulse Rate [bpm]	before 6MWT	EG	77.72	10.97	78.10	11.57	+0.37	-4.34 to 3.58	0.845
		UC	75.17	11.40	80.13	9.98	+4.95	-8.94 to -0.96	0.017*
	after 6MWT	EG	89.10	13.32	90.13	12.85	+1.03	-5.47 to 3.40	0.636
		UC	91.82	14.81	96.04	16.14	+4.21	-10.83 to 2.39	0.199
Distance [m]	after 6MWT	EG	411.65	69.95	441.41	68.38	+29.75	-43.46 to -16.05	0.000*
		UC	445.56	79.17	421.95	67.54	-23.60	1.60 to 45.60	0.036*
Borg fatigue score	after 6MWT	EG	2.51	1.59	2.24	0.91	-0.27	-0.23 to 0.78	0.354
		UC	2.28	0.70	2.82	1.23	+0.54	-0.24 to 1.12	0.030*

Abbreviations: M, mean; SD, standard deviation; UC, Usual Care Group; EG, Physical Exercise Group; SpO_2, Oxygen Saturation; BP, Blood Pressure

after RT. The usual care group (UG) demonstrated a statistically significant increase in HR (before RT), and Borg dyspnea score (above 0.5 point) and a decrease 6MWD (p = 0.05) after radiation treatment.

After RT in both patient groups, a decrease in most blood parameters was observed without anisocytosis (there was a statistically significant increase in the EG). The liver parameters did not change (p = 0.05) in either of the study groups. There was no statistically

Table 3. Effects of Physical Exercise on Serology Outcome Indicators in Prostate Cancer Patients Receiving Radiotherapy

Characteristic		Baseline		After treatment		Change From Baseline to After Treatment		
		M	SD	M	SD	M	95% CI	p
Hemoglobin [g/dl]	EG	8.82	0.76	8.50	0.62	-0.31	0.11 to 0.52	0.003*
	UC	8.79	0.86	8.38	0.73	-0.41	0.17 to 0.64	0.001*
Red blood cells [x10^{12}/l]	EG	4.76	0.48	4.52	0.42	-0.24	0.11 to 0.37	0.000*
	UC	4.71	0.41	4.36	0.38	-0.35	0.21 to 0.49	0.000*
Hematocrit [l/l]	EG	0.42	0.03	0.39	0.02	-0.02	0.01 to 0.03	0.000*
	UC	0.42	0.03	0.39	0.03	-0.02	0.01 to 0.04	0.000*
Anisocytes	EG	12.90	0.80	13.31	1.04	+0.40	-0.76 to -0.04	0.028*
	UC	13.27	0.84	13.47	0.80	+0.20	-0.49 to 0.08	0.159
White blood cells [x10^{9}/l]	EG	7.29	2.13	5.38	1.61	-1.90	1.11 to 2.70	0.000*
	UC	7.36	1.64	5.57	1.11	-1.78	1.26 to 2.21	0.000*
Lymphocytes [x10^{9}/l]	EG	1.92	0.73	1.07	0.95	-0.94	0.42 to 1.26	0.000*
	UC	2.26	0.66	1.13	0.44	-1.10	0.89 to 1.31	0.000*
Neutrophils [x10^{9}/l]	EG	4.47	1.60	3.67	1.18	-0.80	0.25 to 1.34	0.005*
	UC	4.15	1.26	3.47	0.87	-0.68	0.28 to 1.07	0.001*
Monocytes [x10^{9}/l]	EG	0.70	0.21	0.64	0.27	-0.06	-0.02 to 0.14	0.139
	UC	0.70	0.20	0.70	0.17	-0.00	-0.08 to 0.07	0.914
Eosinophils [x10^{9}/l]	EG	0.15	0.08	0.16	0.09	-0.009	-0.06 to 0.04	0.699
	UC	0.24	0.28	0.24	0.19	0.00	-0.13 to 0.13	0.969
Platelets [x10^{9}/l]	EG	228.00	55.71	208.30	40.20	-19.69	4.48 to 34.90	0.013*
	UC	230.29	39.58	211.79	42.99	-18.50	4.61 to 32.38	0.011*

Abbreviations: M, mean; SD, standard deviation; UC, Usual Care Group; EG, Physical Exercise Group.

Table 4. Effects of Physical Exercise on Blood Indicators in Prostate Cancer Patients Receiving Radiotherapy

Characteristic		Baseline		After treatment		Change From Baseline to After Treatment		
		M	SD	M	SD	M	95% CI	p
Urea [mg/dl]	EG	38.28	9.74	33.42	9.93	-4,85	-6.76 to +16.47	0.345
	UC	39.14	11.39	35.21	9.20	-3.92	-0.94 to +8.80	0.104
Creatinine [mg/dl]	EG	0.84	0.21	0.94	0.14	+0.10	-0.03 to 0.23	0.112
	UC	1.00	0.24	0.94	0.22	-0.06	-0.02 to +0.15	0.146
PSA [ng/ml]	EG	3.75	3.09	0.43	0.91	-3.32	+0.07 to 5.58	0.021*
	UC	5.51	6.45	1.61	3,09	-3.89	+0.81 to +6.96	0.016*
AST [U/l]	EG	27.50	8.93	25.50	5.82	+2.00	-4.08 to +8.08	0.436
	UC	27.50	11.38	24.58	7.57	-2.91	2.31 to +8.15	0.245
ALT [U/l]	EG	32.50	15.12	32.00	13.97	-0.27	-8.73 to +9.73	0.894
	UC	34.08	13.73	33.25	14.72	-0.83	-8.28 to 9.95	0.201
Total cholesterol [mg/dl]	EG	212.81	45.84	204.51	44.22	-8.29	-4.78 to 21.37	0.203
	UC	200.66	48.08	196.95	33.84	-3.70	-9.62 to 17.04	0.571
HDL [mg/dl]	EG	51.29	11.65	54.50	11.73	2.79	-0.43 to 6.02	0.186
	UC	54.59	15.27	53.07	13.64	-1.51	-1.85 to 4.89	0.363
LDL [mg/dl]	EG	139.96	46.83	131.81	41.62	-8.14	-0.75 to 17.05	0.071
	UC	135.54	44.05	138.54	39.40	+3.00	-12.98 to 18.98	0.701
TG [mg/dl]	EG	198.61	113.71	188.50	104.19	-10.11	-21.10 to 41.33	0.510
	UC	141.33	63.68	160.66	65.54	+19.33	-56.99 to 18.32	0.299

Abbreviations: M, mean; SD, standard deviation; UC, Usual Care Group; EG, Physical Exercise Group; PSA, prostate specific antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglycerides.

significant changes in renal parameters (urea and creatinine) after the study either. PSA decreased by a statistically significantly amount in both groups after RT with ADT. In the EG, all measured lipid parameters decreased after RT (total cholesterol, LDL, TG), in contrast to the UG (increase in TG and LDL).

The changes in blood and serum parameters in both PCa patients groups are presented in **Table 3** and **4**.

Discussion

Our study suggested that supervised, regular physical activity in high-risk PCa patients during RT improves aerobic capacity, decreases Borg fatigue score after exercise, and corrects lipid parameters. However, the exercise training used in our study did not influence serology outcomes or other blood indicators in patients undergoing RT in either group.

Exercise can contribute to increased physical capacity and can prevent cardiovascular disease in cancer patients [19, 20] These findings support previous research by confirming the safety of and benefits stemming from aerobic exercise during RT. The prescribed exercise duration, frequency, and intensity were matched to possible results, as the American Cancer Society recommends for cancer patients [19]. In a study of PCa patients with home-based aerobic training during RT, Windsor et al. [5] observed that patients in the exercise arm of the study appeared to tolerate their treatment better and reported less severe radiation toxicities. After the study, the authors observed an increase in the distance test and in physical functioning. Kapur et al. [4] concluded that a well-defined exercise schedule appears to reduce the severity of rectal toxicity during RT to the prostate. They observed that patients in the exercise arm of the study appeared to tolerate their treatment better and reported less severe radiation toxicities. After our study, we concluded that regular, moderate physical exercise improves capacity tolerance with a substantial decrease in fatigue (dyspnea) after the 6MWT. Similar results have been observed by other authors [3, 5].

Ionizing radiation causes a series of hematological alterations, especially profound lymphocytopenia during and after the radiotherapy course [22]. In our study, we observed a significant decrease in hemorheology after RT and study results suggested no significant impact of exercise training on these parameters. Segal et al. [2] did not suggest a relationship between exercise groups in hemoglobin or PSA levels in men receiving RT for PCa. Our results are comparative to this result. One reason may be that PSA levels in the usual-care group were higher at baseline and had further to decline during the intervention period. In contrast, another study by Drouin et al. [23] suggested that moderate intensity aerobic exercise (of walking for 20 to 45 minutes, 3 to 5 times per week, at 50% to 70% of measured maximum heart rates) appeared to maintain erythrocyte levels during RT of breast cancer.

Our data shows that supervised exercise training during RT with ADT improved lipid parameters in PCa patients. These parameters (LDL, TG, total cholesterol) were better when compared with patients without physical activity. Similar observations appeared in two other studies [2, 24]. Our results confirmed the influence of physical activity on lipid profile and its role in cardiovascular protection in PCa patients.

However, this supervised, regular exercise training did not cause significant changes in liver and renal functional biomarkers (urea, creatinine, ALT and AST). Total creatine kinase levels depend on age, muscle mass, and physical activity. High levels of serum creatine in apparently healthy subjects may correlate with physical training status [25]. In our patients, we observed an inverse tendency in both groups, but the changes were not statistically significant, therefore, we can assume that this physical training was not a heavy and long burden for this patient group. The levels of biochemical parameters were normal (good state of health people) before and after RT with ADT.

To our knowledge, the present study is the first to provide information on changes in renal and liver function, as well as blood parameters during physical exercise in men with high-risk PCa treated with RT and ADT. Our positive findings of the benefits stemming from a exercise program during RT must be interpreted cautiously because of several study limitations.

The small size of this study is a limitation that restricts statistical power and may explain why some of the observed changes did not achieve statistical significance.

In summary, the regular physical exercises, in high-risk PCa patients, during RT and ADT improves aerobic capacity tolerance with a decrease in Borg fatigue score after treatment. This moderate intensity activity did not influence most serology outcomes, but improved lipid parameters after RT.

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Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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REVIEW PAPER

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Foot arch condition in comparison with the muscular balance of lower limbs in children at school age of 6–14 years

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ABSTRACT

Introduction. Foot arch condition plays an important role in correct setting of lower limbs joints, proper muscles tone and well-being [1]. More and more frequently foot arch deviations affect population of school age children [2, 3]. It is based on anatomic knowledge that abnormal foot arch is strongly connected with the disturbed muscle tone of lower limb. The aim of the presented study is to evaluate the relation between the foot arch condition and muscular balance of the lower limbs.
Material and methods. Children were assessed using the Clarke's angle and indicatory muscles length tests including: quadratus lumborum muscle, hamstring muscle, thigh adductors, piriformis muscle.
Results. Abnormal foot arch was showed by 70% of subject children. The greatest number of muscles length abnormality was observed in quadratus lumborum muscles. There was a statistically significant correlation between right hamstring muscle contraction and abnormal foot arch ($p = 0.011$).
Conclusions. Foot arch alternations increasingly more often appear in the greater number of school–age children. The assessment and therapy of abnormal foot arch should include the examination of muscular balance of the lower limbs.

Keywords: children, flat foot, muscular length, hamstring muscle.

Introduction

The human foot is made up of 26 bones and above 30 articulations. It ensures supporting, shock absorbing and weight-bearing functions [4]. The proper human foot condition has been the center of interest of many researches [5].

Postural abnormalities more frequently affect children and youth at school age [6, 7, 8]. Foot arch defects are ones of the most recognizable postural deviation among children and adolescents [2, 3]. They have differentiated etiologies and causes which are connected with school environment and behavioral habits [5, 9].

Low level of physical activity, leisure time spending passively, invalid nutritional habits, overweight and obesity result in body posture defects [10, 11]. Postural abnormalities are inseparably associated with the changes of muscles length and laxity [12]. The muscular imbalance affects foot arches and results in foot abnormalities, mainly flat foot [13–15]. It should be emphasized that flat foot is the physiological stage during the first decade of life and does not need treatment. It is visible when children begin standing and walking and disappears spontaneously [15]. However, the progress of untreated abnormal foot condition may result in structural changes in the skeletal system, incorrect setting of lower limbs joints and escalating pain [15]. That is why the foot posture should be examined by a specialist in order to exclude pathological conditions.

Aim

The aim of the presented study is to evaluate the relation between the foot arch condition with muscular balance: the length of lower limbs indicatory muscles (quadratus lumborum muscle, hamstring muscle, thigh adductors, piriformis muscle) in children at the age between 6 and 14 years old from the primary school of the Mosina Community.

Material and methods

The study group consisted of 65 healthy school children (40 girls, 25 boys) attending the primary school in the area of the Mosina Community. The age of examined children ranged from 6 to 14 years and the average age was 9 years old and 4 months = 1 years and 7 months. Participants were divided according to age range into 4 groups: < 6 years old (5 children), 7–10 years old (37 children), 11–12 years old (19 children), 13–14 years old (4 children) [16].

Measurement

The pedograph footprints were used to evaluate foot arch of children. They was obtained by placing the child's foot covered with footprint ink on the stretched paper. Based on the obtained footprint, Clarke's angle was calculated as foot arching parameter. Clarke's angle assesses the longitudinal foot arch and is widely used in screening examination [17, 18]. It was constructed by drawing the first medial tangential line of the footprint and the line connecting the first metatarsal head and the acme of the medial longitudinal arch cavity [1, 19].

The results of the Clarke's angle were recorded as "normal" (the Clarke's angle between 42°–52°), "flattened" (the Clarke's angle between 31°–41°) and "flat" (the Clarke's angle below 30°) [19]. All footprints were evaluated by the same person.

Muscles of the lower limbs were assessed with indicatory muscles length tests including: quadratus lumborum muscle, hamstring muscle, thigh adductors, piriformis muscle. The length of quadratus lumborum muscle was assessed while standing when the child slided his/her arm along the outer side of the thigh, without front or back bending. (Norm: The fingers of bending side reach the edge of the knee). Passive straight leg raise test was conducted to evaluate hamstring muscles (Norm: Hip flexion amounts to 80°) and Patrick's Test to evaluate tight adductors (Norm: The flexed knee reaches the level of the straight leg). Piriformis muscle was tested in pronation with flexed knees. The hips were laterally rotated. (Norm: Lateral rotation of hip amounts to 30°) [20].

Protocol and data analysis

Data were analyzed using Microsoft Excel by Microsoft Office 2007 and STATISTICA 8.1. Categorical variables were investigated using Chi^2 Pearson Test and Test Chi^2 Pearson Yates Test. Mann-Whitney U test was used for comparing two independent samples. The correlation coefficient was statistically significant when the p value was less than 0.05.

Results

Foot arch was assessed as normal in 30% of children, flattened in 30% of children, flat in 40% of children (**Figure 1**). In the group of boys flattened and flat foot

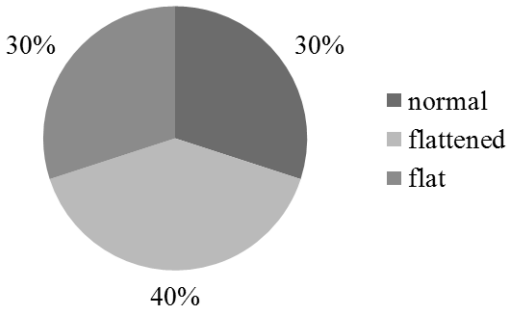

Figure 1. Results of the Clarke's angle

Table 1. The number of shortened indicatory muscles, including the right and left side

	Quadratus lumborum muscle		Hamstring muscles		Tight adductors		Piriformis muscle	
	Left	Right	Left	Right	Left	Right	Left	Right
The number of children	38%	29%	28%	31%	14%	9%	6%	11%

Table 2. The number of correct muscles length, including the right and left side

	Quadratus lumborum muscle		Hamstring muscles		Tight adductors		Piriformis muscle	
	Left	Right	Left	Right	Left	Right	Left	Right
The number of children	62%	71%	72%	69%	86%	91%	94%	89%

Table 3. The relationship between the results of the evaluation of lower limbs muscles tonicity and foot arch

	Quadratus lumborum muscle		Hamstring muscles		Tight adductors		Piriformis muscle	
The number of children with abnormal foot arch	Left	Right	Left	Right	Left	Right	Left	Right
	16	14	17	14	2	5	3	3
p	NS*	NS*	NS*	r**	NS*	NS*	NS*	NS*

** – not significant;*
*** – correlation coefficient*

was presented more often (84%) than in the group of girls (63%).

Tables 1 and **2** illustrate the results of muscle tonicity evaluation.

The measurement of muscles flexibility shows that quadratus lumborum muscles were the most often shortened muscles. In contrast piriformis muscles had the least often tendency to shorten.

Relationship between foot arch and lower limbs muscles tonicity

Comparative analyses were conducted between the results of indicatory muscles length tests (quadratus lumborum muscle, hamstring muscle, thigh adductors, piriformis muscle) and the results of Clarke Index. The obtained results are presented in **Table 3**.

There was a statistically significant correlation between right hamstring muscle contraction and abnormal foot arch ($p = 0.011$). However, there was no significant correlation between left hamstring muscle length and foot arch alterations ($p = 0.10796$).

Discussion

In the past decade flat foot has been an object of many studies and it seems to be a very common postural problem in children [21, 22]. In the present study deviation of proper foot condition occurred in 70% of children. This is consistent with previously study of foot arches condition among children at school age [23, 24]. In the study done by Janiszewska et al., foot arches malformation occurred in more than half of the children [25]. The significantly higher results obtained Brzeska et al, the frequency of flat foot was observed in 78.4% of the research group [26].

Flat foot was more frequent among the group of 7–10-year-old children in the presented research. Literature suggests that the majority of preschool children commonly present abnormality in foot arches [27, 28]. Different results in the presented study can result from very small number of children in the research group who were in preschool age. Walczak and Napiontek have suggested that the occurrence of abnormal foot arches condition among preschool children is connected with a layer of fat tissue covering foot arches which is physiological for this period of the ontogenesis [29]. The foot arches development change over time in close relation to ontogenesis [27]. It is noteworthy that the frequency of flat foot decreases in children over 10 years old. Similar observations were made by Kania – Gudzio and Wiernicka [30]. The age above 10 years refers to improvements in forming foot arches or development of increased foot arches during puberty. The foot spontaneously evolves to the proper physiological shape of the adult's foot at around 10 years of age [27]. In the study performed on the group of children from the Hausa ethnic group of Nigeria, Umar and Tafida diagnosed flatfoot significantly more often in the group of girls than in the group of boys [31]. Opposite to the research by Umar and Talida, in the presented study flatfoot was more common postural alteration among boys than girls. This is consistent with data published by other researchers [15, 32, 33]. The literature suggests that higher incidence of flat foot in boys may be results of the general process of foot morphology and development. The intensive period of the growth of boys feet begins later and last longer [34]. It occurs between 13 and 15 years of age [35].

There are a lot of factors contributing to the development of foot flat, such as BMI, gender, and age [36]. Only few authors take into consideration flat foot and muscular imbalance in their analyses [37, 38]. That is why the main aim of the presented study was to make comparative analysis of length of lower limbs muscles with foot arches condition. The presented study found that there was a significant correlation between right hamstring muscle contraction and abnormal flat foot. This is consistent with the other studies and anatomy of myofascial system [37–40]. There is exists skleletal-myofascial connection between two tapes of: the Spiral and the Lateral Tape, through two tendons: peroneus longus muscle tendon and peroneus brevis muscle tendon. The Spiral Tape passes under the foot arch, creating a loop (stirrup), which is a connection between tibialis anterior muscle and peroneus longus muscle. It joins the foot arch with upper part of a calf.

Myofascial structures of peroneus longus muscle passes through external part of a shank to the caput fibulae, where there is located Connection between peroneus longus muscle with biceps femoris muscle (lateral side of hamstrings muscle group). There is a pronounced fascial connection between caput fibulae and peroneus longus muscle [39]. That is why there is a relationship between contraction of hamstring muscle and flat foot which was presented by Ippalito et al. [40]. They showed in Volumetric magnetic resonance imaging that postero-medial muscular compartments of the leg with flat foot are thinner and shorter than those of the normal leg [40].

It should be noted that, in the future untreated defects in foot arches condition such as flatfoot may play an important role in the development of pain and pathologic conditions of lower limbs [1, 15, 21].

Conclusions

Based on the observation and analysis of the presented study we proposed the following conclusions:

1. Many children at school age suffer from foot arches defects.
2. The assessment and therapy of children with foot arch alterations should consider the examination of muscle tonicity of lower limbs.

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Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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REVIEW PAPER

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Homocysteine – relation to hypertension, age and smoking in patients with newly diagnosed essential hypertension

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ABSTRACT

Introduction. An elevated circulating concentration of homocysteine is associated with an increased risk of coronary, cerebral, and peripheral vascular disease. The purpose of this study was to determine the concentrations of homocysteine in the patients with newly diagnosed essential hypertension and healthy subjects and to analyze the correlation between homocysteine level and the other parameters (age, blood pressure, smoking).
Material and methods. The study group consisted of 18 patients with essential hypertension, 4 women and 14 men (mean age 43 ± 16 years) and 15 healthy volunteers, 8 women and 7 men (mean age 47 ± 10 years). Serum homocysteine was analyzed by FPIA method (Fluorescence Polarization Immunoassay).
Results. The patients with essential hypertension had significantly higher homocysteine concentration compared to control group. No correlation was observed between homocysteine levels and age, diastolic, systolic blood pressure in subjects with essential hypertension. In healthy volunteers, only a correlation between age and homocysteine concentration was found.

Keywords: homocysteine, hypertension, blood pressure.

Introduction

Homocysteine is a sulfur-containing intermediate product in the normal metabolism of methionine, an essential amino acid.

Homocysteine (Hcy) is a product of dietary methionine demethylation, an abundant aminoacid in animal protein. It is present in plasma in four different forms: around 1% circulates as free thiol, 70–80% remains disulphide-bound to plasma proteins, mainly albumin and 20–30% as a dimer homocysteine. Homocysteine is a key determinant of the methylation cycle [1].

Measurement of total plasma or serum homocysteine represents the sum of oxidized and protein bound homocysteine. Homocysteine contains a reactive sulphydryl group that can react with plasma constituents, which may promote oxidative damage. An elevated homocysteine level therefore induces thrombogenicity, causes procoagulant state and promotes the proliferation of smooth muscle cells [2].

Hyperhomocysteinemia is characterized by an abnormally high level (above 15 µmol/L) of homocysteine in the blood (**Table 1**); normal range for plasma homocysteine concentration increases with age (**Table 2**). Other factors influencing homocysteine concentration are genetic factors, drugs, clinical conditions (renal and thyroid dysfunction, cancer, psoriasis, diabetes), lifestyle (alcohol, tobacco, coffee), gender, menopause, muscle mass [3].

Two types of hyperhomocysteinemia, which can be distinguished, are:

- rare severe forms caused by major mutations of genes encoding enzymes responsible for homocysteine metabolism,
- more common moderately elevated homocysteine levels related to genetic and environmental factors [4].

Folic acid, vitamin B12, and B6 deficiency and reduced enzyme activities inhibit the breakdown of homocysteine, thus increasing the intracellular homocysteine concentration [5].

Table 1. Concentration of homocysteine in various stages of hyperhomocysteinemia

	Plasma homocysteine concentration [μmol/L]
Normal range: HPLC immunoassay	 5.0–15.0 5.0–12.0
Moderate hyperhomocysteinemia	16.0–30.0
Intermediate hyperhomocysteinemia	31.0–100.0
Severe hyperhomocysteinemia	> 100.0

Table 2. Normal reference ranges of plasma homocysteine levels for different age groups [years]

	Age 12–19	Age ⩾ 60
Men	4.3–9.9	5.9–15.3
Women	3.3–7.2	4.9–11.6

Numerous retrospective and prospective studies have consistently found an independent relationship between mild hyperhomocysteinemia and cardiovascular diseases or all-cause mortality [1].

Increase in homocysteine level can lead to damage of endothelial cells, decreased flexibility of blood vessels leading to aortic stiffness and to reduction of the speed of blood flow, reduced production of the vasodilator nitric oxide (NO). Therefore, increased plasma homocysteine can promote atherosclerotic disease, including coronary disease, stroke and peripheral vascular disease.

Several epidemiological studies revealed that a 5 μmol increase in plasma homocysteine results in 60% higher prevalence of ischaemic heart disease. Other studies demonstrated that the treatment of hyperhomocysteinaemia reduces atherosclerotic plaque area, thus decreasing the risk of deep vein thrombosis, stroke and ischaemic heart disease [6–7].

High blood pressure is a major risk factor for cardiovascular diseases. Although its etiology has not been fully explained mostly because of as yet unknown genetic variation, multiple nonhereditary factors including dietary and other lifestyle factors have been identified to have important and modifiable influences on blood pressure. Results of several studies suggest that mild increase in serum homocysteine may contribute to elevations in blood pressure [8].

The hypothesis that homocysteine may play a role in the pathogenesis of essential hypertension is based on the fact that homocysteine induces arteriolar constriction, renal dysfunction, increased sodium reabsorption and arterial stiffness. Also, elevated homocysteine is known to increase oxidative stress that causes oxidative injury to the vascular endothelium, diminishes vasodilation by nitric oxide, stimulates the proliferation of vascular smooth muscle cells, and alters the elastic properties of the vascular wall. All these factors are associated with the rise in hypertension. Thus, homocysteine may contribute to blood pressure elevation [9–12].

Little is known about the relation between homocysteine levels and blood pressure in newly diagnosed essential hypertension. Therefore, we have tested the homocysteine concentration in relation to the age, blood pressure and smoking in the patients with newly diagnosed hypertension.

Material and methods

The study was carried out in the Department of Clinical Pharmacology (University of Medical Sciences

Table 3. Clinical characteristic of patients with newly diagnosed essential hypertension and control

	Patients	Control
Number of participants	22 (8F, 14 M)	18 (9F, 9M)
Age [years]	43 ± 16	47 ± 10
Height [cm]	174 ± 11	171 ± 6
Weight [kg]	76 ± 13	70 ± 9
Body mass index [kg/m^2]	24.72 ± 2.22	23.21 ± 1.86
Smoking [n]	12	10
SBP [mm Hg]	158 ± 8	123 ± 7
DBP [mm Hg]	97 ± 5	75 ± 7

in Poznań, Poland) and involved 40 participants (17 females, 23 males; 39 to 65 years; see **Table 3**). The subjects were divided into control group (18 healthy people; 9 females, 9 males) and group of patients with newly diagnosed essential hypertension (22 patients; 8 females, 14 males).

The control group did not show any signs of organ's pathology (especially concerning cardiovascular system, liver and kidney's activity or inflammatory state) in subjective and biochemical examinations (blood morphology, ESR, lipid balance, liver tests, urine analysis) nor in additional examinations (blood pressure measurement). The questionnaire provided information about smoking history and medications.

In a pre-study period and during the study the participants did not take any drugs (or contraceptives in case of women). Nobody was abusing alcohol.

In both groups, a clinical study was conducted, including basic anthropometric measurement used to calculate BMI values. Weight was measured on a balance scale while height was measured in the standing position.

Blood pressure of all subjects was measured twice on the right arm after 5 minutes of rest, using a standard mercury sphygmomanometer. The mean of these 2 readings was used to classify blood pressure according to JNC VII, where hypertension is defined as systolic blood pressure more than 140 mm Hg or diastolic blood pressure more than 90 mm Hg.

Secondary reasons of hypertension and obesity were excluded in the patients with newly diagnosed hypertension.

Patients with coexisting heart failure, ischemic heart disease, peripheral arteries disease (carotid arteries, vertebral arteries or lower limbs arteries), kidney failure (serum creatinine concentration > 115 nmol/l), liver dysfunction (transaminases values 2.5 times higher than normal), diabetes (or disturbed tolerance to glucose) or acute/persistent inflammatory state were not qualified for the research.

The subjects were asked to fast for 10 hours. After all aseptic measures, 6 ml of blood was collected from the antecubital vein while the subjects were sitting up right.

Serum homocysteine concentration was analyzed by immunochemical method with measurement of fluorescence intensity in polarized light (Fluorescence Polarization Immunoassay – FPIA) on IMx analyzer using ABBOTT commercial kits.

Written consent for participation in the study was a mandatory condition for taking part in the research.

Statistical analyses

The all statistical analyses were performed using the CSS STATISTICA program (V 7.0; StatSoft). The mean values and standard deviations were calculated using descriptive module. Before further analyses, normal distribution of the variables was checked with the Shapiro-Wilk test. Variables with abnormal distribution were analyzed by Mann-Whitney Test for comparisons within the groups. The statistical significance was determined at p values below than 0.05.

Results

The patients with essential hypertension had significantly higher homocysteine concentration (15.23 ± 6.41 mmol/L vs. 9.71 ± 3.21; p = 0.001; see **Table 4** and **Figure 1**) as compared to the control group; moreover, 28% of patients had the homocysteine level greater than 15 µmol/l. Additionaly, the homocysteine

Table 4. Homocysteine concentration in patients and control group; * p < 0.05, ** p< 0.001

Homocysteine concentration [µmol/l]	Patients	Control
Everyone	15.23 ± 6.41**	9.71 ± 3.21
Smokers	17.66 ± 7.57*	10.56 ± 3.18
Non-smokers	12.32 ± 2.93	8.63 ± 3.12

Table 5. Correlation coefficients between homocysteine and studied parameters in patients with newly diagnosed essential hypertension and controls; * p < 0.05

Homocysteine		
Correlated parameters	Patients	Controls
age	- 0.09	0.68*
BMI	-0.16	0.31
SBP	-0.15	0.38
DBP	-0.12	0.36

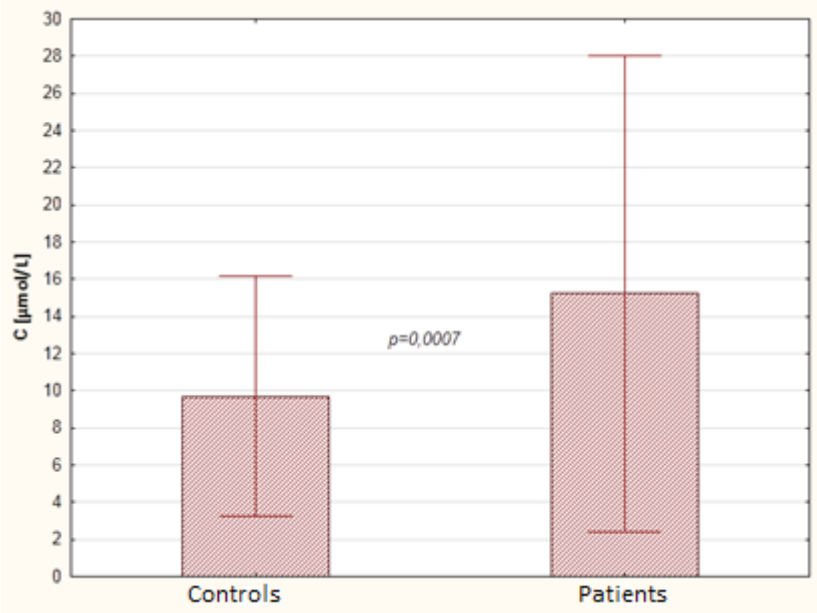

Figure 1. Blood homocysteine concentration in patients with newly diagnosed essential hypertension and controls

concentration in hypertensive smokers was significantly higher than in non-smokers (p = 0.05; see **Table 4**).

No correlation was observed between homocysteine levels and age, diastolic and systolic blood pressure in patients with essential hypertension. In healthy volunteers only the correlation between age and homocysteine concentration was found. The results are summarized in **Table 5**.

Discussion

Homocysteine has been under a lot of speculation since its discovery in 1932. In 1969, an association between homocysteine and cardiovascular disease (CVD) was proposed when it was observed that people with a rare hereditary condition called homocystinuria are prone to develop severe cardiovascular disease in their teens and twenties. By the early 1990's, elevated homocysteine has been considered an independent risk factor for cardiovascular diseases (along with cholesterol and other lipid markers, age, gender, smoking status, obesity, hypertension and diabetes). It was shown that plasma homocysteine is more strongly associated with systolic than with diastolic blood pressure. As a result, it can increase arterial stiffness. However, the results of the studies investigating this hypothesis were inconsistent [13–15].

It is also believed that hyperhomocysteinemia damages endothelial cells, reduces the flexibility of vessels, and adversely affects the process of hemostasis. Additionaly, hyperhomocysteinemia increases the adverse effects of such risk factors as hypertension, smoking, impaired glucose, lipid and lipoprotein metabolism, and can promote the development of inflammation [7].

The meta-analysis performed by Boushey et al in 1995 indicated that an increase of 5 µmol/L was associated with a 60% increase in risk of coronary artery disease in men and an 80% increase in risk in women. Also, a reported 50% increase in cerebrovascular disease was reported. This magnitude of increase in plasma homocysteine was thought to be equivalent to the CVD risk of a 19-mg/dL increase in cholesterol. The European Concerted Action Project also confirmed that the elevated plasma homocysteine was an independent risk factor for CVD, and calculated that an increase of 5 µmol/L was associated with the increase in relative risk for CVD of 1.35. [16–17].

High blood pressure is a major risk factor for cardiovascular disease. It can be influenced by multiple non-hereditary risk factors (including dietetics and lifestyle). Among others, elevated plasma homocysteine may contribute to increase in blood pressure. Moreover, blood pressure may mediate part of the cardiotoxic effect of homocysteine [18].

Several epidemiological studies have examined the relationships between homocysteine and hypertension. Some of these examinations have found significant, although weak, association between plasma homocysteine and blood pressure. Elevated plasma Hcy levels have been consistently reported in hypertensive patients of different age and ethnicity.

The results showed that fasting plasma homocysteine concentrations were significantly higher in subjects with hypertension than in those with normotension (mean +/- SEM, 8.1 +/- 0.6 v 6.8 +/- 0.2 micromol/L; P <.05) [19]. It was shown that essential hypertension in adolescents is associated with lower folate and higher homocysteine levels, and with signs of insulin resistance. Therefore, hypertension in young individuals may be a part of early manifestation of insulin resistance syndrome, and that disturbed folate and homocysteine metabolism may play a role in early stages of hypertension [20–21].

The results of the National Health and Nutrition Examination Survey (NHANES) denoted that homocysteine was shown to have an independent, positive association with blood pressure, stronger in women than in men. A one-standard deviation increase in homocysteine was associated with an increase in systolic blood pressure (SBP) and diastolic blood pressure (DBP) of 0.7 and 0.5 mm Hg in men, respectively, and in women, the increases in SBP and DBP were 1.2 and 0.7 mm Hg, respectively [8]. The comparison carried out by Sutton-Tyrrell et al reported that the level of plasma amino acid rose from 9.7 µmol/L at a SBP <140 mm Hg to 13 µmol/L at SBP >180 mm Hg [22]. Głowinska et al reported higher homocysteine level in hypertensive children [23].

Different results of this correlation were also obtained. Dinavahi et al. corroborated a significant, direct correlation of plasma homocysteine with SBP and DBP in premenopausal women, but not in men. However, when other factors, like age and body mass index were taken into account, no significant correlation was found [24].

Sundström et al. found no major relation of baseline plasma homocysteine level to hypertension incidence or longitudinal blood pressure progression in a large, community-based cohort of nonhypertensive individuals after adjustment for age, sex, and other important co-variates [25].

At vascular level, the continuing exposure to high homocysteine concentration leads to structural and functional changes in the vascular wall. Therefore, endothelial dysfunction seems to constitute a common association between homocysteine, hypertension and atherosclerosis.

There are some direct and indirect mechanisms by which high homocysteine exerts detrimental vascular effects. In a healthy endothelium NO rapidly reacts with homocysteine to form S-nitrosohomocysteine, which constitutes a protective mechanism. High homocysteine level can compromise NO bioavailability, inhibiting its regulatory endothelial vascular action thus leading to injury and dysfunction.

Increased homocysteine levels may also decrease NO bioavailability by increasing asymmetric dimethylarginine (ADMA) an analogue of L-arginine which acts as a competitive inhibitor of endothelial Nitric Oxide Synthase (eNOS). ADMA can also promote superoxide generation via uncoupling eNOS enzyme activity [26–27]. Reactive oxygen species (ROS)-induced oxidative stress represents a hallmark in endothelial dysfunction. A significant increase in reactive oxygen species at vascular levels in animal models and human hypertensive subjects has been described. Results of many studies strongly suggest that both processes, a diminished NO availability and increased ROS production coexist, constituting a common feature in human hypertension [28].

In hypertension, the increase in arterial wall thickness and the loss of elasticity over time results in the increase in pulse wave velocity, a direct measure of arterial stiffness. This change is reflected in gradual fragmentation and loss of elastin fibers and accumulation of stiffer collagen fibers in the media that occurs independently of atherosclerosis. Similar results are seen with an elevated level of homocysteine known as hyperhomocysteinemia, which increases vascular thickness, elastin fragmentation, and arterial blood pressure [29–30].

Cardiovascular diseases remain the main cause of mortality in industrialized countries and have become increasingly prevalent in developing countries. The risk of developing cardiovascular disease is mainly attributable to a number of known risk factors, which are in first instance hyperlipidemia, hypertension, smoking and diabetes mellitus. Smoking is one of the most important risk factors for cardiovascular diseases. Components of tobacco smoke cause physiological and morphological changes in endothelial cells and increase the concentration of many negatively acting substances, including homocysteine [31].

The association between elevated homocysteine concentrations and coronary, cerebral or peripheral artery disease was investigated in numerous epidemiological studies with either retrospective or prospective study design. The elevated homocysteine is known to increase oxidative stress that causes oxidative injury to the vascular endothelium, diminishes vasodilation by nitric oxide, stimulates the proliferation of vascular smooth muscle cells, and alters the elastic properties of the vascular wall. All these are associated with the

rise in hypertension. Thus, homocysteine may contribute to blood pressure elevation. Higher levels of homocysteine in patients with primary hypertension may be an argument for introducing the evaluation of this amino acid concentration in clinical examinations.

It was observed that plasma homocysteine concentration in normotensive children of hypertensive parents is elevated before the development of hypertension. Therefore, homocysteine level may be predictive of the subsequent development of hypertension in such patients [32].

According to Catena et al., plasma homocysteine was significantly greater in hypertensive patients with evidence of carotid plaques than patients without carotid plaques. Moreover, carotid intima-media thickness progressively increased across quartiles of plasma Hcy levels and was independently related with age, blood pressure, C-reactive protein, and Hcy levels. These results suggest the role of elevated plasma homocysteine in the development and progression of carotid atherosclerosis [33].

Conclusions

1. Significantly higher homocysteine concentration was observed in the group of patients with primary hypertension, compared to the control group.
2. Significantly higher homocysteine concentration was observed in the group of smoking patients with primary hypertension, compared to the non-smoking patients.
3. No correlation was observed between homocysteine levels and age, diastolic, and systolic blood pressure in patients with essential hypertension.

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Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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REVIEW PAPER

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An introduction to genetic and epigenetic changes in prostate gland – implications in efficacy of phytotherapy of benign prostatic hyperplasia and prostate cancer

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ABSTRACT

The usage of classical pharmacological treatment of prostate diseases causes the risk of a number of side effects therefore the researchers are looking for new pharmacologically active molecules, including those contained in the plant extracts. The most widely studied is the lipido-sterolic extract from Serenoa repens (saw palmetto), water extract from Camellia sinensis (green tea) and several cruciferous vegetables. The molecular mechanisms underlying of the development and the progression of prostate disorders, especially benign prostatic hyperplasia (BPH) and prostate cancer (PC), remain still poorly understood. The development of pathologically changed prostate cells proliferation involves many factors, including genetic alterations, such as mutations, and epigenetic changes, appear to contribute to the transformation and progression of prostate cancer. In this paper we suggest that the knowledge of epigenetic modifications presented in this paper introduces the new point of view concerning the possibility of action of plant substances used in prevention and symptomatic treatment of BPH and prostate cancer. Thus, identification of the epigenetic modifications involved on the one hand in the development and progression of BPH / PC, on the other influencing the efficacy and safety of potential phytotherapeutics will be helpful in identifying its novel therapeutic strategy.

Keywords: prostate disorders, phytotherapy, epigenetic modifications.

Introduction

An efficient pharmacotherapy of prostate diseases, especially benign prostatic hyperplasia (BPH) and prostate cancer (PC), are extremely important aspects of the modern medicine because of the increasing number of illnesses as a result of the increase in life expectancy and the fact of aging of population [Cohen and Rokhlin, 2009].

Prostate cancer is a common illness, which leads to death among men all over the world [Cohen and Rokhlin, 2009]. The molecular mechanisms underlying of the development and the progression of prostate cancer remains still poorly understood. Genetic alterations, such as mutations, and epigenetic changes, defined as heritable changes in gene expression that occur without changes in DNA sequence, appear to contribute to the malignant transformation and progression of prostate cancer [Li et al., 2005]. Benign prostatic hyperplasia (BPH) is a common, progressive with age, disease which occurs in approximately 50% of men aged about 60 years old, 90% of men aged about 90 years of age resulting in a serious medical and social problem. The

consequence is progression of urethral stenosis, and thus the appearance of troublesome symptoms in the lower part of the urinary tract (LUTS – lower urinary tract symptoms), affecting the quality of life [Fong., 2004].

Currently, the first-line pharmacological treatment options in men with BPH, moderate to severe LUTS and as adjuvant therapy of androgen-dependent prostate cancer the 2nd classes of drugs, mainly alpha-adrenergic blockers (alpha-blockers) and 5alpha-reductase inhibitors (5-ARIs; azasteroids – finasteride, dutasteride) are indicated [Gravas et al., 2010; Nickel et al., 2010; Elterman et al., 2010]. Steroid 5a-reductase 2 (SRD5A2) catalyzes the conversion of testosterone to the more potent androgen, DHT, in the prostate [Luo et al., 2003]. Decreased expression of SRD5A2 has been observed in prostate cancer. Many authors indicate that there is an association of prostate cancer with reduced 5a-reductase enzymatic activity as a result of remarkably decreased expression of the SRD5A2 gene [Luo et al., 2003].

The usage of classical pharmacological treatment causes however the risk of a number of side effects i.e.: orthostatic hypertony, tachycardia (alpha-blockers), abnormal ejaculation, decreased volume of ejaculate, erectile dysfunction (5-ARIs) [Lepor, 2011]. They have a proven impact on reducing prostate size by modifying the concentration of DHT acting on epithelial cells and core. Influence of the size of the prostate 5 alpha reductase inhibitors results of blocking the enzyme – 5-alpha steroid reductase – converting the testosterone (T) to dihydrotestosterone (DHT), the active form responsible for the growth, proliferation and development of the prostate and to facilitate atrophy and apoptosis [Gravas et al., 2010]. However, the necessity of their long application and, consequently, the emergence of a number of side effects forced researchers to search for new pharmacologically active molecules, including those contained in the plant extracts.

Phytotherapy of BPH and PC

Indeed, herbal medicines are used the most: they have an established position in prophylaxis and symptomatic treatment and prevention of urological diseases [Lowe et al., 2008; Macdonald et al., 2012; Kim et al., 2012; Morán et al., 2013]. One of the best examples of modern phytotherapy is the treatment of BPH [Azimi et al., 2012; Morán et al., 2013]. Although plant materials used for the prevention and treatment of BPH, PC and LUTS are based mainly on the tradition of natural medicine, there is growing number of preclinical and clinical studies attempting to determine the safety and efficacy of preparations of plant origin [Kim et al., 2012; Morán et al., 2013] . The most popular is Serenoa repens (saw palmetto) [Macdonald et al., 2012], also Pygeum africanum (african plum), Cucurbita pepo (pumpkin), as well as Urtica dioica, Zea mays, Secale cereale, Hypoxis rooperi (south African grass) and other [Wilt et al., 2000; Cristoni et al., 2000; Steenkamp, 2003; PDR, 2007; Lowe, 2008; Dedhia et al., 2008; Wehrberger et al., 2012; Azimi et al., 2012; Kim et al., 2012]. For them mostly antiproliferative effects, normalization of steroid hormones disturbance (antiandrogenic, antiestrogenic effects), decreasing the level of androgens transporter proteins (mostly SHBG), relaxation of smooth muscle in prostate, bladder gland and anti-inflammatory or antioxidant properties have been described [Lowe et al., 1999; Wilt et al., 2000; Dreikorn, 2002; PDR, 2007; Azimi et al., 2012; Morán et al., 2013].

Their molecular mechanism of action is still not fully understood, however, it is well known that it is mainly multidirectional, and is based primarily on the change in the enzymatic activity of 5α-steroid reductase, aromatase or lipooxygenases enzymes, protein growth factors, androgen (AR), estrogen (ER) receptors, as well as α-adrenergic and muscarinic receptors activities or by the promoting of the proteins regulating apoptosis in proliferating prostate cells (i.e. caspases, Bax/Bcl2 proteins) [Madersbacher et al., 2008; Dedhia et al., 2008; Kujawski et al., 2010; Wehrberger et al., 2012; Kim et al., 2012; Morán et al., 2013]. Several in vitro studies confirmed especially the inhibitory activity of plant extracts on steroid 5α-reductase activity, resulting in a decrease of DHT in the cells, without causing the increased expression levels of prostate specific antigen (PSA) [Delos et al., 1994; Di Silverio et al., 1998; Hsieh et al., 2002; Hill et al., 2004;]. Also Yang and coworkers confirmed the induction of apoptosis in several cancer cell lines (LNCaP, PC-3, DU145) by extract from fruits of Serenoa repens manifested by increased expression of proteins "guardians of apoptosis" p21waf1 and p53 [Yang, 2007]. In our studies we have shown that administration of Epilobium angustifolium aqueous extract caused a decrease in the level of expression of selected genes involved in the pathology of BPH in prostate lobes in rats and a small pro-androgenic effect [Kujawski et al., 2010; Kujawski et al., 2013; Kujawski et al., 2014; not fully-published data], the natural chemopreventive agents to counteract these cancer-related epigenetic alterations by influencing the activity or expression of DNA methyltransferases and histone modifying enzymes.

According to current state of knowledge chemopreventive agents that target the epigenome in cancer cells include so-called micronutrients (folate, retinoic acid, and selenium compounds), butyrate, polyphenols from green tea, apples, coffee, black raspberries, and other dietary sources, genistein and soy isoflavones, curcumin, resveratrol, dihydrocoumarin, nordihydroguaiaretic acid (NDGA), lycopene, anacardic acid, garcinol, some compounds from Allium species and several cruciferous vegetables, including indol-3-carbinol (I3C), diindolylmethane (DIM), sulforaphane, phenylethyl isothiocyanate (PEITC), phenylhexyl isothiocyanate (PHI), diallyldisulfide (DADS) and allyl mercaptan (AM), cambinol, and relatively unexplored modulators of histone lysine methylation. Up to date the above data are based mainly on in vitro assays, and results of animal models or human intervention studies are limited that demonstrate the functional relevance of epigenetic mechanisms for health promoting of BPH and/or PC preventive efficacy of natural products [Ho et al., 2011; Gerhauser, 2013; Chiam et al., 2014]. Furthemore, literature data does not provide so far the complex information on the molecular mechanism of action of extracts based on above mentioned plant substances in prostate gland.

Molecular aspects of phytotherapy of prostate disease progression

Epigenetics refers to the study of heritable changes in gene expression without any changes in DNA sequence. Epigenetic processes involve three interacting molecular mechanisms: DNA methylation, modification of histones in chromatin and RNA-mediated regulation of gene expression [Peedicayil, 2006]. These patterns are known to be reversible and vary with age as well as varying from tissue to tissue, since an individual has multiple epigenomes. The importance of epigenetics in clinical medicine has been increasingly appreciated, both in the pathogenesis of single-gene disorders and common diseases. Among the common diseases, except of cancer, little is known at present regarding the role of epigenetics in the pathogenesis of these diseases [Feinberg, 2007]. One of the most important epigenetic aberration is DNA methylation, which is the addition of a methyl group to the 5′-carbon of cytosine in CpG sequences, catalyzed by DNA methyltransferases (DNMTs). Methylcytosine residues are often found in short stretches of CpG-rich regions (i.e., CpG islands) that are 0.5–2 kb long and found in the 5′ region of approximately 60% of genes [Gardiner-Garden and Frommer, 1987].

The development of pathologically changed prostate cells proliferation involves many factors, including genetic alterations, such as mutations, and epigenetic changes, appear to contribute to the transformation and progression of prostate cancer. Studies on cell lines focused so far on a number of genes, but the most important for the epigenetic changes and their relation to the development of prostate proliferation, especially the prostate cancer, are classified into four groups: hormonal response genes (androgen receptor (AR), Steroid 5-α-reductase type 2 (SRD5a2), estrogen receptors (ERs): ER alpha (ESR1) and ER beta (ESR2)), cell cycle control genes (RB1), cell invasion genes (CD44, adenomatous polyposis coli (APC)) and DNA damage repair genes (glutathione S-transferase Pi (GSTP1)).

In our opinion it is very important to investigate whether plant extract has a direct impact on changes in the level of methylation and expression of selected genes, which are modifications influencing development of prostate cancer.

Hormonal response genes

In the literature there are few studies evaluating the changes in the methylation profile of gene sequences in the pathogenesis of hormone-dependent prostate in vivo. There is a growing number of in vitro studies concerning this problem. Current state of knowledge presents a positive relationship between epigenetics modifications and expression level of androgen receptor (AR) gene in prostate cancer development. Aberrant hypermethylation in the AR promoter region may play a critical role in AR expression in rat prostate cancers [Takahashi et al., 2002]. It is well known that hypermethylation is a potential transcriptional regulatory mechanism in prostate cancer in an approximately ~ 1.5-kb region of CpG island in the AR gene in the AR expression-negative cell lines Du145, DuPro, TSUPRI, and PPC1 [Jarrard et al., 1998], which has been not detected in non-tumor prostate epithelial cells [Jarrard et al., 1998; Nakayama et al., 2000]. Takahashi et al. have not observed the AR mRNA expression in any of the rat prostate cancer model or human cancer cell lines (PLS10, 20, and 30) and all of the examined rat prostate and seminal vesicle cancers demonstrated hypermethylation at these CpG sites [Takahashi et al., 2002]. The authors showed that methylation of CpG sites at -312, -274, -9, and -1 nucleotides upstream of the transcriptional initiation site correlated well with AR mRNA expression in rat prostate.

Therefore, in our opinion, the biological changes of the methylation-mediated AR inactivation in prostate

occurring in BPH as a potential source of cancerous development and the effects of several plant extracts on the level of expression of AR gene and methylation status of CpG sites at -312, -274, -9, and -1 nucleotides upstream of the transcriptional initiation site should be investigated.

Estrogen receptors (ERs) A and B have been identified in normal and cancerous prostate tissue [Karr et al., 1979]. Hypermethylation of cytosine-rich areas in promoters of ESR genes seem to be associated with the transcriptional inactivation and also has been detected frequently in cancer [Esteller et al., 2001]. ESR receptor genes appear to be inactivated by CpG methylation in prostate cancer tissue and cell lines [Sasaki et al., 2002]. The extent of ESR1 and ESR2 promoter methylation is significantly less in the BPH than in prostate tumors [Li et al., 2000], indicating that prostate cancer induces ESR gene hypermethylation.

The analysis of the literature data found no studies on changes in the level of methylation in the promoter of ESR1 and ESR2 in the prostate of the rat. The most analyzed regions in these genes are ER1 promoter region (spanning –186 to –45 upstream of transcription start site) and ER2 promoter region (spanning –625 to –392, important regulatory region) [Doshi et al., 2011]. It is very important to evaluate the methylation status of these regions in ESR1 and ESR2 genes in, for example, in vivo BPH pathogenesis model under the influence of herbal extracts.

There are many published data concerning the methylation status of SRD5A2 gene in BPH and PC and its influence on the expression level as well as if any of the bioactive compounds of analyzed in this project extracts would be responsible for the epigenetic regulation of studied hormonally dependent genes. Blanchard et al analysed the promoter region of the 5alpha-reductase type 1 gene and observed that the high percentage of G + C which accounted for 61.3% of the nucleotides in the region from –1000 to +1; numerous CpG were present, and the CpG observed/CpG expected ratio was 0.76 for this 1 kb window. Further, within a 500 bp region (from –500 to +1), no less than 11 potential Sp1 binding sites (GC and GT boxes) were found with sequence homology very close to the Sp1 consensus sequence. Based on the above mentioned results, in our opinion, the evaluation of such 500 bp length fragment against changes in the level of methylation, their relationship to the level of gene expression of 5alpha-reductase and changes in methylation and expression under the influence of crucial in the phytotherapy of BPH/PC plant extracts could be a very important step enabling an assessment of their mechanism of action and therefore safety and efficacy with a comedication with currently used drugs of first choice.

DNA damage repair genes

Hypermethylation of genes involved in DNA damage repair, has been reported in prostate cancer [Li et al., 2005]. Methylation changes in the glutathione S-transferase P1 (GSTP1) promoter are the most frequent alterations in prostate cancer development. Many scientific data indicated that hypermethylation of GSTP1 is involved in intracellular detoxification reactions and in loss of gene expression. Hypermethylation has been found in >90% of prostatic carcinomas, including early disease stages, and has not been detected in normal tissues [Goessl, 2000.].

The comparison between methylation status of DNA damage repair genes in benign prostatic and prostate cancer cell lines showed that frequencies of the methylation level in prostate cancer were higher than in BPH [Yamanaka et al., 2003].

A literature analysis shows no studies on the analysis of methylation in the promoter of the gene GSTP1 in rat prostate. Therefore, in order to obtain a better understanding of the molecular mechanism of action of plant extracts in prostate gland, we postulate, based on studies presented by Nakayama et al., that the investigation of the methylation status of promoter region (a region extending in human GSTP1 from a pentad [ATAAA]n repeat sequence located at -414 of the GSTP1 transcription start site to an area between +296 and +625 of the gene) and the expression level of this gene could be a very promising course .

Cell cycle control genes

Retinoblastoma (RB1) protein plays a crucial role in regulation of the cell cycle and has been identified in many tumor types as a tumor suppressor gene [Lee et al.,1987]. According to our knowledge there is only one published study resulting in the information that loss of RB1 expression correlated with homozygous deletion or promoter hypermethylation in histologically heterogeneous prostate carcinomas, which indicate that hypermethylation is correlated with the loss of gene function [Konishi et al., 2002]. The analysis of the literature data found no studies on the analysis of methylation in the promoter of the gene of RB1 in rat prostate. Therefore, the authors based on studies presented by Stirzaker et al. investigated the methylation status of the promoter region in 185–206 bp upstream

of the initial codon and contains putative binding sites for transcription factors RBF-1, Sp1, ATF and EF2, in gene RB1 in rat prostate. It is very important to verify the methylation status and expression level of RB1 in the pathogenesis of BPH in prostate gland (in vivo models, clinical samples), especially under the influence of selected plant extracts. Such mechanism of action in prostate cells is still unknown. Therefore, these aspects should be more complex studied.

Cell invasion genes

The APC gene encodes a protein with multiple cellular functions and interactions, including roles in signal transduction in the Wnt-signalling pathway, mediation of intercellular adhesion, stabilization of the cytoskeleton and possibly regulation of the cell cycle and apoptosis [Fearnhead and all., 2001]. Methylation in APC promoter was associated with an increased risk of prostate cancer–specific mortality and promoter methylation in APC was identified as a marker for prostate cancer progression [Richiardi et al., 2009]. However such studies were performed so far in in vitro cell cultures, thus there are no published data concerning the methylation status of APC gene in prostate gland. CD 44 is also an important integral membrane protein receptor, playing a crucial role in cell-cell and cell-matrix interactions, and has been implicated in tumor growth and migration [Naor et al., 1997]. Some studies demonstrated that loss of CD44 expression correlates with methylation and was associated with increased grade and pathological stage of prostate cancer [Verkaik et al., 2000]. Such mechanism of action of plant origin bio-active compounds in prostate cells is still unknown. Therefore, these aspects require further comprehensive studies.

Conclusions

Epigenetic modifications ongoing in pathophysiologically changed prostate gland in response of pathophysiological process may be a crucial factors in the progression of prostate diseases (BPH, PC). Although the pharmacological activities of plant origin substances have been described, their above mentioned epigenetic mechanisms of action are still unknown. The authors suggest that the knowledge of epigenetic modifications presented in this paper introduces the new point of view concerning the possibility of action of plant substances used in prevention and symptomatic treatment of BPH and prostate cancer. Thus, identification of the epigenetic modifications involved on the one hand in the development and progression of BPH/PC, on the other influencing the efficacy and safety of potential phytotherapeutics will be helpful in identifying its novel therapeutic strategy.

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REVIEW PAPER

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Clinical trials in relapsing-remitting multiple sclerosis (a new proposal for dealing with basic problems and restrictions)

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ABSTRACT

The natural course of multiple sclerosis is characterized by a high variability of pattern, relapse rate and different progression indices. They also present a dramatic impact on the interpretation of treatment trials. Older reports, based on uncontrolled observations are therefore of little value. Currently it is generally accepted that a proper treatment trial should be double blinded and, although probably controversial, that it should be compared with a group of MS patients treated with placebo. Such an approach would be easily acceptable to prove the effectiveness of recently discovered disease modifying drugs. We know that current standard methods of therapy with interferon beta or glatiramer acetate are able to decrease the relapse rate at least by one third, to elongate the intervals between the relapses and to decrease the progression indices. Currently MS is considered a generalized degenerative disease. The lesions are persistent, therefore immunomodulatory treatment has to be started as early as possible. An alternative approach, somewhat suggestive for the use of placebo trials, seems to be a comparison of proposed new drug therapy group with a group of patients treated with a generally accepted reference drug, such as interferon beta or glatiramer acetate, including all clinical and MRI measures.

Keywords: multiple sclerosis, clinical trial, placebo, pathophysiology, reference drug.

Introduction

Multiple sclerosis (MS) is a subacute inflammatory disease invading the central nervous system (CNS) of young adults, with presumably T cell-mediated autoimmune pathogenesis. The incidence and prevalence of MS is significantly higher in females than in males. The reason for such a gender difference remains still unknown.

The natural cause of MS is characterized by variability of pattern, relapse rate and progression indices. In many patients there is a tendency to improve even without treatment. These facts create a dramatic impact on the interpretation of treatment trials. Older reports, based on uncontrolled observations were critically presented in a monography on therapeutic claims in MS [1] and are of less current value. The spontaneous remissions were often presented with great enthusiasm in older reports. Nevertheless, when the same method was replicated in other locations, results seemed not better than no therapy. This possibly means that results of the proposed new therapy were only describing the natural course of MS.

It would not be difficult to judge the efficiency of a new treatment when the marked improvement would occur in all cases or more importantly, when completely stopping the progress of the disease. So far, newly proposed methods were partially effective and therefore may only be evaluated by proper methods of clinical trials.

The first currently accepted rule is that the trial should be double blinded, from both patients and phy-

sicians in order to avoid over enthusiasm with regard to improvement. In a good clinical trial, the team has to be strictly divided into the evaluating and treating physicians who are to guard the safety of the patients during the trial.

Multicentre trials usually provide a larger number of subjects and avoid bias related to ethnic or geographical differences. MS is most common in Caucasians living far away from the equator in the northern and southern hemispheres.

The results of treatment are evaluated by clinical and imaging techniques, such as structural MRI (magnetic resonance imaging), with or without gadolinium enhancement. In multicentre trials standardization based on exact definitions is required. The clinical status of the patient is assessed using a scoring system, e.g. the functional systems score (FS) included in the expanded disability status scale (EDSS) Kurtzke [2]. Intrarater and interrater reliability of the EDSS scale is sufficient. Aditionally, the multiple sclerosis functional composite (MSFC) is used [3]. MSFC test, the ambulation (timed 25-foot walk), arm function (Nine-Hole Peg Test) and cognitive function (Paced Auditory Serial Addition Test (PASAT)). EDSS and MSFC scoring, when repeated in the course of trial, make it possible to establish whether or not the disease is progressing, has stopped or ameliorates. The use of scales yields also a possibility to establish objectively the occurrence of relapses or clinical deterioration. It is, actually, more complicated than a mere scoring matter, i.e. McDonald's diagnosis criteria are used.

A strict definition of relapse is used, i.e. the appearance of a new neurological abnormality or worsening of an existing one, separated by at least 30 days from a the previous attack A relapse should be confirmed by an evaluating physician. Confirmation is based on increase by 1point in each of two functional systems (FS) or by 2 points in one.

Based on the agreed definitions and scoring systems, the number of relapse-free patients, the number of patients with treated relapses, the annual relapse rate and the number of patients with sustained clinical worsening is calculated within each trial.

Clinical results are compared with MRI lesions seen on repeated scans. The main MRI parameters include the number and volume of new and newly enlarging T2 hyperintensive and T1 hypointensive lesion [4, 5]. A measure of the volume of brain parenchymal fraction as an indication of brain atrophy is also performed [6].

One point in the planning of clinical trials in MS is both exciting and controversial at the same time. Should the group of patients randomized to the effective studied drug be compared with another one, receiving placebo. Such an approach was easily acceptable to prove the effectiveness of disease modifying drugs. We know that current standard methods of therapy with interferon beta or glatiramer acetate are capable to decrease at least by one third the relapse rate, to elongate the intervals between the relapses as well as to decrease the progression indices based on the EDSS scale [7]. The impact of drug therapy on MRI lesions in the course of the process is even greater [4]. The idea of placebo-controlled clinical trials in MS was thoroughly discussed by international task force groups of clinicians, statisticians and ethicist and conclusions were published in 2001 and 2009 as a commonly reached consensus [8]. The conclusion was, that the placebo controlled clinical trials are acceptable from an ethical point of view, but only when the respective patients are fully aware of the overall available treatment procedures, or when they have already failed on previous therapy, or else, when for various reasons, they have no access to other treatment methods [9, 10].

However, quite recently, some new facts have been revealed in the field of pathophysiology of MS, which shadowed with dark clouds the placebo-controlled clinical trials in MS.

Currently, MS is considered as a degenerative disease of the brain and spinal cord in which an inflammatory process leads early not only to demyelinating lesions but also to an injury of axons, to neuronal loss and cerebral atrophy [6, 11]. The lesions are persistent, which is the reason why immunomodulatory treatment has to be started as soon as possible [12].

Early axonal lesions were seen using immunohistochemical methods, such as the SMI-22 antibody detection method [13]. Normal, myelinated axons contain phosphorylated neurofilaments which are not stained bySMI-22. In acute MS lesions, in normal appearing white matter and even in the grey matter of post-mortem MS specimens, numerous dendrites with non-phosphorylated neurofilaments are found, thus showing axonal dysfunction. The presence of terminal axonal ovoids is proof of tissue transsection. Similar observations were made using other axonal protein marker of amyloid precursor.

Magnetic resonance tomography using T1-weighted (T1W) and T2-weighted (T2W) protocols shows atrophy of the grey matter, particularly within the cerebral cortex. It occurs early in the evolution of MS, and is of high clinical significance, triggering several symptoms and signs of the disease [14–17]. Recently evi-

dent severe lesions were also found within the lower motor neurons of the spinal cord. Early immunomodulatory treatment can stop the progress of degenerative changes [12]. This does not happen in patients receiving placebo, even when an active treatment would later follow. To avoid ethical criticism related to the delay of active treatment, some protocols have limited the duration of the study to a 6-months period, followed by open label treatment with the active drug only for the patients group.

An alternative approach, indicative of the rational value of placebo trials seems to be a comparison of the patients group receiving the new drug with a matched group treated with the reference drug of general acceptance e.g. interferon beta or glatiramer acetate, and assessment of subsequent clinical and MRI changes.

I understand that the presented point of view stands against the accepted dogma about the value of placebo control trials in MS. However, the numerous limitations raised by ethical principles against the use of placebo in MS trials, raised by Ethics Committees protecting those principles may lead to a creation of special groups of treated patients, that are not identical with the total cohort of MS patients. In this sense, the use of reference treated groups may even improve the validity of results.

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REVIEW PAPER

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TAZ oncogene as a prognostic factor in breast cancer

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ABSTRACT

Breast cancer is the most frequently diagnosed cancer in females and one of the main causes of cancer related deaths. Breast cancer in the metastatic stage is related with poor prognosis. Metastasis is the process whereby cancer cells travel to and colonize distant sites through the lymphatic system or bloodstream, which usually indicates a poor prognosis. The metastatic cascade involves several molecular and cellular interactions and different signaling pathaways. Recently the Hippo signaling has emerged as an important regulatory pathway in cancer. The Hippo target protein TAZ has been reported as a novel oncogene that may have important role in the development of breast cancer. Its overexpression promotes cancer stem cell formation and epithelial–mesenchymal transition in many human cancers. In breast TAZ seems to play a critical developmental role and in breast cancer is one of the factors involved in therapeutic resistance and clinical relapse. Herein, we review the biological functions of TAZ and summarize the current knowledge and opportunities for therapeutic intervention in this field.

Keywords: breast cancer prognosis, cancer stem cells, Hippo pathway, targeted therapy.

Introduction

Breast cancer is one of the most common cancers in women and a cause of around a half million deaths annually worldwide (World Cancer Report 2014. International Agency for Research on Cancer, World Health Organization. 2014.). Numerous studies show that the pathogenesis of breast cancer is highly related with a small population of cells with stem cell characteristics which are named cancer stem cells (CSC). One of the important regulators of CSC phenotype is the Hippo tumor suppressor signaling pathway.

Hippo pathway is a main downstream effector regulating cell proliferation, tumor formation, apoptosis and organ size [2]. Highly conserved in its structure, the pathway was originally described in Drosophila melanogaster as an organ size regulator. It consists of a cascade of kinases, MST and LATS in mammals, that phosphorylate the effector proteins YAP and TAZ, thereby controlling their nucleo-cytoplasmic localization and functions. MST1/2 phosphorylate and activate the kinases LATS1/2, which in turn phosphorylate YAP and TAZ, leading to their cytoplasmic retention [3]. Inactivation of the MST and LATS kinases results in nuclear accumulation of oncoproteins YAP and TAZ and subsequent activation of target genes, many of them involved in cell proliferation and epithelial-to-mesenchymal transition (EMT).

TAZ (transcriptional co-activator with PDZ-binding motif) is a component of the pathway, which structurally shares 50% sequence identity with its paralog –YAP. TAZ contains one WW domain, which allows it to bind other partners that exhibit proline-rich modules named PY motifs [4]. In the cell nucleus TAZ binds with the primary partners which are TEA domain (TEAD) transcription factors. TAZ function as transcriptional co-activator for them, therefore overexpression of TAZ induces

cell transformation and tumor-forming ability in mammary epithelial cells [5]. The deactivation of the Hippo pathway, which leads to upregulation of YAP and TAZ is frequently observed in many human cancers.

TAZ is reported to confer cancer stem cell-related traits on breast cancer cells and a novel oncogene that may have important roles in the development of breast cancer.

Herein, we review of the Hippo pathway in human, and discuss the biological functions of TAZ and summarize the current knowledge and opportunities for therapeutic intervention in this field.

The Hippo pathway

Numerous studies show that the Hippo pathway plays a key role in tumorigenesis. Its deregulation is frequently observed in many human cancers. Deactivation of Hippo pathway leads to YAP and TAZ translocation into nucleus which in turn promotes transcription of downstream genes by forming complexes with transcription factors. Conversely, Hippo pathway activation, cause YAP and TAZ sequestration in the cytoplasm and further degradation.

The Hippo pathway also plays an important role in normal mammary gland development. TAZ is reported to regulate the number and complexity of branches within mammary gland and determine the fate of basal and luminal cells in mammary glands [6, 7]. The Hippo pathway was reported to be highly transcriptionally deregulated but only in case of basal-like subtype of breast cancer.

TAZ in human cancer

A range of cancers displays high levels of TAZ or YAP expression such as carcinomas of the lung, thyroid, ovarian, colorectal, prostate, pancreas, esophagus or liver [8–10]. This high level of expression has been associated with shorter patient overall survival [11–13].

In non-small cell lung cancer TAZ protein was reported to be expressed in 66.8% cases (that is 121/181), which also significantly correlated with poorer differentiation and short survival [14]. In gastric cancer samples, Yue et al reported TAZ positive expression in 77.4% (113 out of 146) and especially higher expressed in signet ring cell carcinoma [15]. The level of TAZ mRNA could also be a prognostic marker in colon cancer progression [11]. This data indicates that TAZ may become a worthy target for therapeutic intervention in future.

TAZ in breast cancer

TAZ is overexpressed in ~21.4% of breast cancer samples, particularly these fulfilling the histopathological criteria of poor differentiation or high grade [5]. Cordenonsi et al.. has previously reported that, CSCs are indeed enriched in high-grade breast cancers (i.e., poorly differentiated tumors classified as G3 by histopathological criteria) when compared to well-differentiated, low-grade (G1) tumors [16]. That fact suggests that TAZ plays a critical role in the migration, invasion, and tumorigenesis of breast cancer cells.

Chan et al. observed that f2- to 3-fold gain of function (by overexpression) in MCF10A cells increased the migratory and invasive properties such as loss of cell adhesion and increased cell mobility of the cells. Furthermore, the overexpression resulted in morphologic change from an epithelial to a fibroblast like appearance indicating that TAZ may take part in regulation of EMT/MET events in breast epithelial cells. The strict mechanism of this action is vague as the expression level of E-cadherin in MCF10A and MCF7 cells is not significantly altered by either overexpression or knockdown of TAZ. In turn, the knockdown of TAZ using short hairpin RNA retard anchorage-independent growth in soft agar in MCF7, although it is not sufficient to enable MCF10A cells to grow into sizeable colonies in this assay and tumorigenesis in nude mice [5].

Cordenonsi et al. indicate that loss of cell-polarity determinant Scribble is one of the responsible factors to induce EMT in breast epithelial cells. TAZ forms a complex with Scribble, and its loss disrupts the inhibitory association of TAZ with MST and LATS. This findings reveals that cell polarity may control of Hippo pathway [17]. Lei et al. reported that constant TAZ expression stimulates cell proliferation with an increase in the S-phase cell population [2]. Furthermore TAZ negatively correlates with disease-free survival in patients with breast cancer [18].

The tumorigenic properties of TAZ may be due to WW and PDZ-binding domains, which play a crucial role in self-renewal of mammary tissue. WW domains of TAZ act through interaction with PPXY motifs of some transcriptional factors. For instance the Runx transcription factors (Runx1, Runx2 and Runx3 that are involved in carcinogenesis and cancer metastasis. Both YAP and TAZ can potentiate Runx activity through WW-PPXY interaction [19]. Moreover, transduction of MCF10A cells with the WW domain mutation results in significantly fewer colonies in soft agar assay in comparison to the wild-type TAZ [20].

The highly conserved PDZ-binding motif localized in the C-terminus of TAZ also confers mammary tumour formation potential. It localizes TAZ into discrete nuclear foci and is essential for TAZ-stimulated gene transcription. Loss of the PDZ-binding motif is reported to abrogate tumour formation in vivo.

The main targets of the YAP and TAZ belong to the TEAD protein family (TEAD1-4) and they function as regulators of cell contact inhibition, EMT, oncogenic transformation and apoptosis inhibition. Li et al. demonstrated that silencing of TEAD1, TEAD3 and TEAD4 by RNAi as well as disruption of TAZ-TEAD binding resulted in completely abrogation of CSC-like traits driven by TAZ [21]. TAZ has also been reported to interact through the WW domain with the PPXY motif of the Kruppel-like factor 5 (KLF5). By this means TAZ protects KLF5 from WWP1-mediated ubiquitination and further degradation, which in turn leads to breast cell proliferation and tumorigenesis [22, p. 5]

Relation to cancer stem cells

The Hippo transducer TAZ confers CSC-related traits on breast cancer cells such as the ability to drive tumorigenesis and contribute to maligancy, therapeutic resistance and clinical relapse. The expression level of TAZ is higher in breast CSCs than that in differentiated breast cancer cells. Expression of cell surface markers is of some assistance in distinguishing the tumorigenic from the nontumorigenic cancer cells. In breast cancer the $CD44^{+}CD24^{-}$ phenotype confers the tumorigenic properties. Serial passaging of this tumorigenic subpopulation generated new tumours containing additional $CD44^{+}CD24^{-/low}Lineage^{-}$ tumorigenic cells as well as populations of nontumorigenic cells present in the initial tumor. TAZ has been determined to be required for the maintenance of the $CD44^{high}/CD24^{low}$ antigen phenotype [5, 23, 24]. Moreover, the $CD44^{high}/CD24^{low}$ subpopulation characterized by a lower in vitro proliferation rate compared to parental cells and resistance to chemotherapy [24].

TAZ induces chemoresistance

Numerous studies show the association between TAZ and chemoresistance of breast cancer [25, 26]. For instance, elevated TAZ levels lead to resistance to the first- line chemotherapeutic drug in breast cancer- Taxol [26]. TAZ contributes to Taxol resistance by inducing the transcription of TAZ targets Cyr61 and CTGF. Lai et al. has knockdowned genes Cyr61 and CTGF by short hairpin RNA in MDA-MB231 in breast cancer cells, what reversed Taxol resistance in these cells. Activation of the Cyr61/CTGF promoters and induction of Taxol resistance was driven by the interaction of TAZ with the TEAD family of transcription factors . Hence the TAZ-TEAD-Cyr61/CTGF signalling pathway is an important modifier and a therapeutic target of the Taxol response in breast cancer cells.

TAZ as prognostic factor

Recently TAZ expression has been linked with HER-2 positivity and the pathological complete response [27]. Patients with low TAZ tumors exhibited pathological complete response rate of 78.6% whereas patients with high TAZ tumors -57.6% ($p = 0.082$). Vici et al. suggest that the level of TAZ assessed by means of immunohistochemistry predicts pathological complete response in 61 patients receiving neoadjuvant chemotherapy and trastuzumab, with both molecular subtypes – Luminal B and HER2-positive.

TAZ as potent therapeutic target in cancer

TAZ has been proposed as a viable target in cancer therapy. The possible therapeutic strategies may include various strategies such as YAP/TAZ-TEAD interaction or upstream kinases [28–31]

Targeting the actin cytoskeleton with the F-actin destabilizers (Atrunculin A/B, Cytochalasin D, Blebbistatin) inhibits nuclear export of TAZ in Drosophila, therefore may be effective in control of tumour growth. [32, 33]. The Hippo pathway is also regulated by G-protein-coupled receptor (GPCR) signalling which can either activate or inhibit the Hippo-YAP pathway depending on the coupled G protein [34]. Ligands like 1-phosphophate (S1P), serum-borne lysophosphatidic acid (LPA), sphingosine thrombin and protease-activated receptor agonists signal through G12/13 coupled receptors, therefore inhibiting the Lats1/2 kinases, and activating TAZ [19]. Therefore, development of agonists mimicking LPA or S1P may be another therapeutic option [35–37]. Therefore, GPCRs are very prominent candidates for anti-cancer drug target [38].

It was also reported that TAZ may be activated by Wnt/βcatenin pathway. Active signalling leads β-catenin to detach from destruction complex and inhibit TAZ degradation, therefore maintaining integral β-catenin destruction complex abrogate TAZ. XAV939, G244-LM and G007-LK are tankyrase inhibitors restor-

ing the integrity of the complex enabling TAZ degradation [39].

Another possible strategy to inhibit nuclear localization and activation of TAZ is through metabolic cues [40]. The mevalonate–YAP/TAZ pathway is required for proliferation and self-renewal of breast cancer cells therefore it is crucial to develop mevalonate pathway inhibitors. Oncogenic cofactor mutant p53, which is the most frequently mutated in breast cancers, promotes transcription factor SREBP activity which in turn increases the levels of mevalonic acid. Mevalonate is a precursor for geranylgeranyl diphosphate (GGPP) which promotes membrane localization and activity of Rho GTPase, that leads to YAP/TAZ nuclear localization and activation. Inhibition of HMG-CoA reductase by statins or bisphosphonates blocks YAP/TAZ nuclear translocation and further transcription. Also, inhibition of geranylgeranylation of Gβγ and RhoA enhances phosphorylation of MST1/2 and LATS1, inhibits the activation of TAZ, and reduces the breast cancer cell migration [41].

Habbig et al. has determined that NPHP4, a known cilia-associated protein and an upstream regulator of TAZ, acts through interaction with LATS1, therefore inhibiting TAZ and YAP phosphorylation [42]. Similarly, NPHP9, competitively binds with TAZ, translocating TAZ into nucleus [43]. It has been shown that the knockdown of both of the proteins inhibits the breast cancer cell proliferation. These findings indicate new ideas for designing anti-YAP/TAZ drugs.

Recently Frangou et al. found that desatinib, a multi-target kinase inhibitor, may modulate TAZ-mediated pro-tumorigenic transcriptional programs [24]. In their study, Dasatinib inhibited the anchorage-independent growth of TAZ-M#1 cells in soft-agar assay and reduced self-renewal as measured by mammosphere formation. FACS analysis showed that TAZ-M#1 cells treated for 24 hours with Dasatinib were depleted of $CD44^{high}/CD24^{low}$ subpopulation, a phenotype associated with cancer stem cells. Conversely the $CD44^{high}/CD24^{high}$ breast cancer subpopulation remained viable. These results indicate that Dasatinib selectively eradicates the TAZ driven breast cancer initiating cells. Further research show that MDA-MB-231 cells treated with Dasatinib injected into SCID mice demonstrate a decreased tumor formation.

Conclusion

The development of breast and breast cancer is determined by multiple, genetic and environmental factors. The crosstalk between signalling pathways, as well as the strict upstream regulators and downstream target genes of the Hippo pathway has not been completely understood. Examination of the Hippo signalling seems to be important in designing more effective cancer therapeutics, especially targeting TAZ.

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Conflict of interest statement

The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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REVIEW PAPER

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Clinical assessment of inflammatory bowel disease activity: a critical overview

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ABSTRACT

Crohn's disease (CD) and ulcerative colitis (UC), which belong to the group of inflammatory bowel diseases (IBD), are chronic inflammatory conditions of the gastrointestinal (GI) tract. Over the last eighty years the overview of IBD has evolved, along with disease symptom recognition, hypotheses on etiology and recommendations for clinical treatment. This review focuses on the clinical aspects of IBD throughout the years and discusses the most recent and future concepts in IBD diagnosis.

Keywords: disease activity index; disease classification; endoscopic and histopathological changes.

Introduction

Crohn's disease (CD) and ulcerative colitis (UC), which belong to the group of inflammatory bowel diseases (IBD), are chronic inflammatory conditions of the gastrointestinal (GI) tract. IBD affect up to 5–10% of worldwide population and currently their etiology remains unknown, although several factors, such as dysregulation of the immune and nervous system functions and changes in gut microflora have been suggested [1].

CD was first mentioned in 1903 by Leśniowski [2] and the full description of the disease was provided by Crohn in 1932 [3]. CD may reveal at any age, but most commonly its symptoms are observed in 15 to 30-year-old patients. A consecutive, small peak of CD incidence is also reported in patients between 40 and 60 years of age. The main manifestations of CD are GI lesions, which are most frequently observed in distal part of ileum, caecum and all parts of the large intestine, but not in rectum. Furthermore, upper parts of the digestive system may be affected as well. Other symptoms of CD include abdominal pain, escalating after the meal and localized in the region of umbilicus or in right iliac fossa. Fever, lasting a few days or even weeks, nausea and vomiting, diarrhea and weight loss may also occur. Moreover, there are frequent manifestations associated with CD outside the GI tract, such as arthritis, iritis, skin lesion and primary sclerosis cholangitis (PSC).

UC was first described in 1875 by Wilks and Moxon [4]. The onset of UC may reveal at any age, but most frequently it occurs in 15 to 40 year old patients, with second peak observed between 50 and 80 years of age. Contrary to CD, UC may be restricted only to rectum, or to rectum and colon. Moreover, while in CD non-inflamed parts of intestine are observed, the inflammation in UC is more continuous. The main symptoms of UC are bloody diarrhea (up to 20 stools per day), rectal urgency and rectal tenesmus. UC may also manifest by abdominal pain associated with defecation and localized in left iliac fossa. In UC, the extra-intestinal manifestations, similar to those seen in CD patients, are observed.

In this review we give a comprehensive outline of clinical, endoscopic and histological indices used in IBD classification and critically discuss by comparing their theoretical usefulness to practice. We also sug-

gest further directions in the design of IBD indices based on clinical experience with those currently used. The experimental results discussed in this review were obtained from a systematic literature search carried out by consulting electronic scientific databases, including MEDLINE, SCOPUS and Web of Science, electronic editorial networks, such as BMJ, Blackwell, Elsevier, Karger, Nature Publishing Group, Springer, and literature distributors. The scientific papers were selected according to the time span ranging principally from 2000 to present.

Disease classification throughout the years

Crohn's disease

As it was shown in numerous studies, CD is a heterogeneous entity (for review, see: [5, 6]) and thus it is usually divided into several subgroups to facilitate the work of both, a clinician and a scientist. Clinician's benefits from a multistage disease classification include simplified assessment of disease prognosis and a relatively easier choice for a successful patient therapy. In case of scientists, a multiscale classification may facilitate the research on understanding the pathomechanisms of the disease.

The Rome classification for CD, established in 1991, included anatomic location and behavior (inflammatory, fistulising, stenotic) of the disease in the GI tract, extent of lesions and operative history. As it turned out to be insufficient and had not been widely used, international Working Party met in Vienna in 1998 to adjust and to update the classification with the most recent findings [7]. New classification contained the age of onset of the disease; changes to the disease behavior and location were also made (**Table 1**).

In 2001, Louis et al. showed, using inter alia phenotype-genotype analyses that the location of lesions is the most reliable component of CD classification, while its behavior varies as the disease progresses [8]. Subsequently, in 2005 the National Working Party presented in Montreal a modified Vienna classification [9]. An overall division based on age at diagnosis, disease location and behavior remained unchanged, but several new features were introduced (**Table 1**). To begin with, the group of the youngest patients was included in the age of onset and thus the former A1 category was split into A1, which now indicated the age of onset before 16 years and A2, from 17 to 40 year old patients. As for location, the L4 modifier was introduced to allow CD in the upper GI tract coexist with disease manifestations in other parts of the digestive system. Additionally, the perianal disease modifier has been included in the behavior section.

It is well known that pediatric patients suffering from any disease need special approach and no exceptions should be made for IBD. Several weaknesses involving pediatrics patients affected by CD were found in the Montreal classification. Thus, pediatric experts met in Paris in 2011 to adjust the existing classification to be more suitable for the youngest patients without influencing the adult assessment [10], in particular as regards the age of diagnosis. The A1 category was now divided into A1a, which stands for age <10 years and A1b (10>17 years old) (**Table 1**). As regards location, the upper GI disease (L4) was rearranged to proxi-

Table 1. Current classifications of Crohn's disease

	Vienna	Montreal	Paris
Age of diagnosis	A_1, below 40 years A_2, above 40 years	A_1, below 17 years A_2, between 17 and 40 years A_3, above 40 years	A_{1a}, between 0 and 10 years A_{1b}, between 10 and 17 years A_2, between 17 and 40 years A_3, above 40 years
Location	L_1, ileal L_2, colonic L_3, ileocolonic L_4, upper GI tract	L_1, ileal L_2, colonic L_3, ileocolonic L_4, separated upper GI tract[a]	L_1, disease in 1/3 of distal ileum L_2, colonic L_3, ileocolonic L_{4a}, upper GI tract disease proximal to ligament of Treitz* L_{4b}, upper GI tract disease distal to ligament of Treitz*
Behavior	B_1, non-stricturing, non-penetrating B_2, stricturing B_3, penetrating	B_1, non-stricturing, non-penetrating B_2, stricturing B_3, penetrating p perianal disease modifier	B_1, non-stricturing, non-penetrating B_2, stricturing B_3, penetrating B_2B_3, penetrating and stricturing disease p perianal disease modifier
Growth retardation			G_0, no growth retardation G_1, growth retardation observed

**L4 modifier can be added to L1, L2, L3 if co-exists with upper GI tract disease.*

mal to the ligament of Treitz (L4a) and distal to the ligament of Treitz, above the 1/3 distal ileum (L4b). In addition, stenosing and penetrating disease could be classified concurrently as B2B3 category. However, the most important was the inclusion of growth retardation in the classification (G0 if negative, G1 if positive).

The comparison between Rome, Montreal and Paris classifications is shown in **Table 1**.

Ulcerative colitis

The UC classification was taken into consideration for the first time by Montreal Working Party and – based on the disease extent and severity – three subtypes have been proposed: ulcerative proctitis (distally to the rectosigmoid junction), left sided UC (distally to the splenic flexure) and extensive UC (proximally to the splenic flexure), known now as pancolitis, when the whole colon is involved in inflammation [9]. Five years later, during a meeting in Paris, a small – but crucial for children patients – change has been made based on the observation made by Van Limbergen et al. [11], who showed that in young patients the extensive involvement of the colon is significantly more frequent compared to the adults (82 vs. 48%, respectively). It was decided that the hepatic flexure will define whether the involvement of the colon is extensive, but not complete or whether the entire colon is affected by the disease.

Current UC classification based on the disease severity assumes the existence of four grades of the disease – remission, mild, intermediate and severe. The symptoms evaluated in UC include bowel movements, heart rate, temperature, hemoglobin level, erythrocyte sedimentation rate, CRP level and the presence of systemic illness.

Establishment of inflammatory bowel disease indices

Crohn's disease indices

Several scales have been used in IBD for the assessment of disease severity, patient's condition, treatment effects, and future clinical approach. In CD, the most common and widely used disease scale is Crohn's Disease Activity Index (CDAI), established by Best et al. in 1976 [12]. Eight parameters are characterized when calculating CDAI: number of liquid stools, presence of abdominal pain, patient's activity, occurrence of extra-intestinal manifestations, administration of antidiarrheals by patient, palpable abdominal mass, hematocrit (HTC), and body weight. Each parameter has its numerical range and – in case of first three – the numbers are summed over 7 days. The final CDAI value below 150 indicates remission, 150 – 450 means active disease and values above 450 are an indication of severe form of CD. Despite its usefulness, CDAI was criticized for a long period of time needed for its assessment, which may be complicated in contemporary practice. Therefore in 1980 Harvey and Bradshaw [13] decided to simplify the index by excluding the laboratory data and the information about the antidiarrheals.

The necessity of establishing a disease scale for young patients resulted in a creation of a pediatric version of CDAI (PCDAI) in 1990 [14]. Three sections were created, i.e. patient's history of the past 7 days, laboratory tests, and clinical examination, in which original CDAI parameters were integrated, unchanged. Additionally, erythrocyte sedimentation rate, albumin concentration, presence of perirectal disease, weight and height have been included. In 1995, Ryżko and Woynarowski [15] presented their version of PCDAI, where weight and height were substituted with Cole index and HTC with hemoglobin concentration. Similarly to CDAI, each parameter in PCDAI is scored for 0, 5 or 10 points, where maximum is 100. A lower PCDAI value indicates better prognosis.

Finally, the efforts were made to create the index for perianal manifestations of CD, which could help directing the surgical approach towards an accurate treatment of the disease. The scoring system proposed by Pikarsky and collaborators in 2002 [16] proved to correlate well with the results of the procedures in patients with perianal CD.

The comment of the clinician

The CDAI / PCDAI indexing system is widely used due to its simplicity and the easiness of collecting the data necessary to calculate the index value. Some may criticize the lack of information about the use of antidiarrhoeal, but in most cases it does not influence further treatment of the patient and therefore no improvement can be necessary.

Ulcerative colitis indices

As summarized in **Table 2**, a wide range of indices have been created for assessing the severity of UC. This was mainly triggered by the need of evaluating the effectiveness of each of the newly created drugs in clinical trials, beginning with steroids and concluding with the latest biological agents.

Table 2. A summary of ulcerative colitis indices

Variable	Index					
	Powell-Tuck	Rachmilewitz (CAI)	Lichtiger	SCCAI	Mayo	UCSS
Number of stools	< 3/24 h = 0 3–6/24 h = 1 > 6/24 h = 2	< 18/week = 0 18–35/week = 1 36–60/week = 2 > 60/week = 3	0–2/24 h = 0 3–4/24 h = 1 5–6/24 h = 2 7–9/24 h = 3 > 10/24 h = 4	1–3/24 h = 0 4–6/24 h = 1 7–9/24 h = 2 > 9/24 h = 3	normal = 0 1–2 more than normal = 1 3–4 more = 2 > 5 more = 3	Similar to Mayo
Stool consistency	normal = 0 semiformed = 1 liquid = 1					
Nocturnal diarrhea			absent = 0 present = 1	1–3 = 1 4–6 = 2		
Urgency of defecation			absent = 0 present = 1	if hurry = 1 if immediately = 2 if incontinence = 3		
Need for antidiarrheal drugs			no = 0 yes = 1			
Blood in stool	no sign = 0 trace = 1 more than a trace = 2	none = 0 little = 2 a lot = 4	none = 0 in < 50% of stools = 1 in ⩾50% of stools = 2 in 100% of stools = 3	traces = 1 occasionally = 2 usually = 3	none = 0 streaks of blood in stool visible = 1 obvious blood in stool visible = 2 blood passes alone = 3	Similar to Mayo
Abdominal pain or cramping	no abdominal pain = 0 with bowel actions = 1 more continuous = 2	none = 0 mild = 1 moderate = 2 severe = 3	none = 0 mild = 1 moderate = 2 severe = 3			
Nausea or vomiting	absent = 0 present = 1					
Abdominal tenderness	none = 0 mild = 1 marked = 2 rebound = 3		none = 0 mild, localized = 1 mild to moderate, diffused = 2 severe or rebound = 3			
Pyrexia	< 37.1 = 0 37.1–38 = 1 > 38 = 2	37–38 = 0 > 38 = 3				
Extra-intestinal manifestation	none = 0 mild on 1 site = 1 severe or mild on 2 sites = 2	if any of these appears: iritis, erythema nodosum, arthritis = 3 / each		if any of these appears: arthritis, pyoderma gangrenosum, erythema nodosum, uveitis = 1 / each		
General condition	no impairment = 0 impaired, able to continue activities = 1 activity reduced = 2 unable to work = 3		perfect = 0 very good = 1 good = 2 average = 3 poor = 4 terrible = 5	very good = 0 fair = 1 poor = 2 very poor = 3 terrible = 4	generally good = 0 fair = 1 poor = 2 terrible = 3	Similar to Mayo
Physician's global assessment		good = 0 average = 1 poor = 2 very poor = 3			from normal to severe = 0–3	from quiescent to severe = 0–3
Laboratory tests		ESR > 50 in 1st h = 1 ESR > 100 in 1st h = 2 Hemoglobin < 100g/l = 4				

ESR, erythrocyte sedimentation rate

The first clinical index for UC activity, the Truelove and Witts Severity Index, was established in 1955 [17]. The scale included six variables: amount of stools per day, blood in stools, body temperature, pulse rate, hemoglobin concentration and erythrocyte sedimentation rate (ESR). One or two stools per day without blood, normal temperature and heart rate, hemoglobin above 11 g/dl and ESR below 20 mm/h defined clinical remission. The Truelove and Witts Severity Index was principally qualitative and it distinguished mild, moderate and severe disease. Its main drawback was that the improvement in patient's condition could not reflect the improvement in index and vice versa, what shows the advantage of the quantitative over qualitative scales. Of interest, Truelove and Witts also created the first 3-point endoscopic scale. Based on a sigmoidoscopic assessment, the following scores were attributed: 1, normal or near normal mucous membrane (slight hyperemia or slight granularity is observed), 2, improved and 3, no change or worse.

The Powell-Tuck Index, named also as the St. Mark's Index, was created in 1978 [18]. Ten clinical variables were included in this scale: number of stools, stool consistency, abdominal pain, associated anorexia, nausea or vomiting, general health, extra-intestinal manifestation in eyes, joints, mouth and/or skin, abdominal tenderness, body temperature and blood in stool (**Table 2**). The Powell-Tuck Index also includes a three-point endoscopic scale (0–2 points), which was added in 1982, describing hemorrhagic intensity in the sigmoid colon. The final score in this index varies from 0 to 22 points and the remission is defined by the score of 3 points and less [19].

In 1987, Schroeder et al. [20] introduced the Disease Activity Index (also known as the Mayo Score or Mayo Clinic Score) (**Table 2**). The score ranges from 0 to 12 and consists of four features with maximum points of 3 each: stool frequency, rectal bleeding, proctosigmoidoscopy score and PGA (Physician's Global Assessment). PGA depends on three subscores and the patient's well-being assessment (which is not, however, included in Mayo Score's final score). Feagan et al. [21] further modified the scale by excluding the endoscopic score and including the patient's functional assessment to obtain the ulcerative colitis clinical score (UCCS) (**Table 2**).

In the same year, Sutherland et al. [22] developed the Ulcerative Colitis Disease Activity Index, in which four variables were included: stool frequency, rectal bleeding, mucosal appearance and physician's assessment of disease activity. Assessment of mucous membrane included friability (1 or 2 points), exudation and spontaneous hemorrhage (3 points).

In 1988, Rachmilewitz et al. [23] established a scoring system including clinical symptoms (medical history and physical examination) and endoscopic findings (colonoscopy). When establishing the Clinical Activity Index (CAI, **Table 2**), seven parameters were characterized: number of stools per week, presence of blood in stools and abdominal pain or cramp, general patient's condition, body temperature, extra-intestinal manifestation of UC and results of the laboratory tests, such as erythrocyte sedimentation rate and hemoglobin concentration. An endoscopic index, also developed by Rachmillewitz et al., was based on the assessment of granulation scattering reflected light, vascular pattern, vulnerability of mucosa and mucosal damage, which included the presence of mucus, fibrin, exudate, erosions and ulcers. The index score of 4 points or less defined remission. CAI is now widely used, especially to confirm effectiveness of new therapies in UC.

In 1992, Seo et al. [24] have made efforts to create another quantitative evaluation of the disease's severity based on Truelove and Witt's classification. Among 18 clinical, laboratory and endoscopy variables, 5 have been proven to significantly correlate with the disease severity, i.e. blood stools, bowel movements, ESR, hemoglobin and serum albumin. The index was calculated as follows: 60 × amount of bloody stools + 13 × bowel movements + 0.5 × ESR + 4 × Hb − 15 × albumin + 200 and the values <150 and >200 refer to mild and severe activity, respectively, with moderate activity located between these two. The newly developed Activity Index showed crucial advantages comparing with previously created scales: the calculations were not cumbersome, could be used for repeating the evaluations, chosen variables were not too invasive to patients and the index was shown to correlate well with endoscopic findings [25].

The Lichtiger Index, which was another modification of Truelove and Witts index, was designed by Lichtiger et al.in 1990 [26] (**Table 2**). Eight features were taken into account: frequency of bowel movements, nocturnal bowel movements, number of blood-stained stools, fecal incontinence, abdominal pain or cramping, general well-being, abdominal tenderness and patient's need for taking antidiarrheals. With the maximum score of 21, the scale is evaluated again in two consecutive days. The final score below 10 means a clinical response to the drug.

The Sigmoidoscopic Inflammation Grade Score was developed by Lémann et al. in 1995 [27]. It is

a four-point scale, in which normal mucous membrane receives 0 points, edema and/or loss of mucosal vascular pattern and granularity – 1 point, induced bleeding on examination (friability) – 2 points and spontaneous hemorrhage, visible ulcers obtained 3 points.

Walmslay and colleagues aimed at developing a scoring system that will suit for daily practice. In 1998, they adopted some features of the Powell-Tuck Index and – using additional scores (sigmoidoscopic assessment with the Baron scoring system, nocturnal defecation and urgency of defecation) and multivariable regression analysis – developed the Simple Clinical Colitis Activity Index (SCCAI) with six variables [28] (**Table 2**). Of note, instead of general health question from Powell-Tuck Index, a general well-being score from the Harvey-Bradshaw index for CD was introduced. The SCCAI scoring system does not require any of the laboratory or endoscopic assessment and therefore seems perfect for the activity assessment even by the general practitioner.

Since the need to perform repeated endoscopies, the existing scales for the assessment of UC described above were acceptable for adult, but not suitable for pediatric patients. Therefore, in 2007 Turner et al. [29] provided the Pediatric Ulcerative Colitis Activity Index (PUCAI). Six variables were chosen as representative: abdominal pain, rectal bleeding, stool consistency, number of stools per day, presence of nocturnal stools and limitation of the activity. With the maximum score of 85, the authors suggested to correlate the response to the therapy with the following changes in PUCAI: small response ⩾10 points, moderate response ⩾20 points and large response ⩾35 points.

The comment of the clinician

Unlike the CDAI, which turned to be the gold standard in assessing disease activity in CD, we lack a fully validated indexing tool in UC. The only exception is the PUCAI, which underwent rigorous evaluations by its authors and may be regarded as superior to other indices [30, 31]. In line, Turner et al. made efforts to compare the existing noninvasive disease activity indices in UC and to elect the most valid ones [32]. The study showed a significant prevalence of three indices over others: SSCAI, PUCAI and, in part, the Mayo score, as it appeared to be strong only in three of four categories chosen in study. As for the PUCAI- even if it was initially constructed with children in mind, the index can be used successfully for evaluating the disease activity in adults, as it includes none of children-specific parameters.

IBD endoscopic indices

Endoscopy is a diagnostic tool, which enables identification of lesions in the GI tract and evaluation of disease progression. It is also used to distinguish CD and UC from enterocolitis with known etiology. Another advantage of endoscopy is the possibility to obtain bioptic samples for histological examination.

Using endoscopy as an imaging tool, Maratka [33] distinguished five types of endoscopic changes in CD: 1) aphtoid stadium with erosions and slight ulcerations surrounded by normal-looking mucous membrane, 2) ulcerative stadium, in which ulcerations with irregular border are surrounded by almost normal mucosa, 3) polypoid stadium with pseudopolyps – fragments of inflamed mucous membrane located between altered, ulcerative surround, 4) cobblestoning stadium, in which surface of mucous membrane resembles cobblestone due to edematous mucosa that is separated by linear ulcerations, and 5) constrictive stadium with stenosis caused by fibrosis of intestinal wall (**Figure 1**).

In 1989, Mary and Modigliani [34] created the Crohn's Disease Endoscopic Index of Severity (CDEIS) (**Table 3**). Bowels were divided into five parts: 1) rectum, 2) sigmoid and left colon, 3) transverse colon, 4) right colon and 5) ileum. In each segment of the bowel, lesions were assessed endoscopically and scored, based on their depth, extent and ulcerative surface; the presence of stenosis with or without ulcerations was also evaluated. The total CDEIS score is a sum of parameters described above and, similarly to other indices, an increased score signified a more severe CD manifestation. However, CDEIS is nowadays regarded as time and labor-consuming and unsuitable in daily practice due to its complexity.

In 2004, Daperno et al. [35] developed a Simple Endoscopic Score for CD (SES-CD). Partition of bowels into five segments is maintained in this scoring system and lesions are given from 0 to 3 points depending on their intensity (**Table 4**).

Capsule endoscopy (CE) was invented in 2000 and was approved by the American Food and Drug Administration FDA in 2001. It became an important element of diagnosis of lesions in the small intestine over the last 10 years. Nowadays, CE is used in case of CD and tumors of small intestine suspicion, as well as a persistent GI bleeding, an ambiguous iron deficiency anemia, chronic abdominal pain and polyposis syndrome. CE allows the visualization of mucous membrane and shows small ulcerations of mucosa, that are not visible in other screening [36–38].

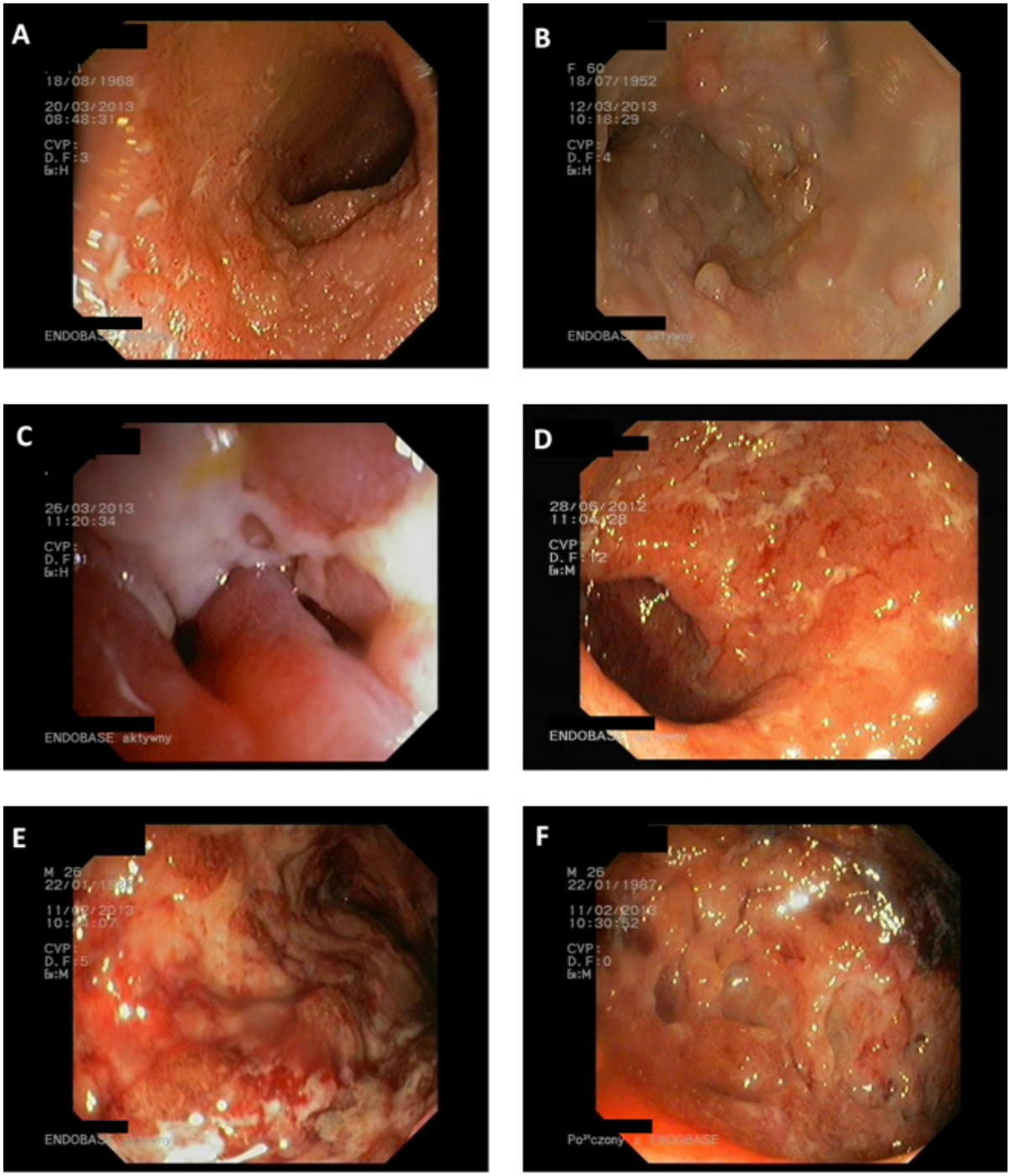

Figure 1. Endoscopic images of histopathological changes in the gastrointestinal tract in Crohn's disease (A-C) and ulcerative colitis (D-F). Crohn's disease: A. Edematous mucous membrane with slight ulceration covered by fibrin in distal part of small intestine. B. Pseudopolyps in sigmoid colon. C. Stenosis of colon at a level of splenic flexure with extensive ulceration covered by fibrin. Ulcerative colitis: D. Hemorrhagic stadium in sigmoid colon – edematous mucous membrane, redness and friability with flat erosions covered by fibrin. E. Ulcerative stadium – flat ulceration covered by fibrin. F. Polypoid stadium – several deep ulcerations covered by fibrin and pseudopolyps

Table 3. Format of calculation of the Crohn's Disease Endoscopic Index of Severity (CDEIS)

	Rectum	Sigmoid and left colon	Transverse colon	Right colon	Ileum	
Deep ulceration	0 or 12 pt	0 or 12 pt	0 or 12 pt	0 or 12 pt	0 or 12 pt	Total 1
Superficial ulceration	0 or 6 pt	0 or 6 pt	0 or 6 pt	0 or 6pt	0 or 6 pt	Total 2
Surface of CD lesions	0–10 cm	0-10 cm	0-10 cm	0-10 cm	0-10 cm	Total 3
Ulcerative surface	0–10 cm	0-10 cm	0-10 cm	0-10 cm	0-10 cm	Total 4
Total 1+Total 2+ Total 3+ Total 4 = A A/number of occupied parts (1-5)= B Presence of ulcerative stenosis: 0 or 3 pt = C Presence of non-ulcerative stenosis: 0 or 3 pt= D B+C+D=CDEIS						

Table 4. Simple endoscopic score for Crohn's disease (SES-CD)

Variable	0	1	2	3
size of ulceration	none	aphthoid ulcerations (diameter: < 0.5 cm)	large ulcerations (diameter: 0.5–2 cm)	very large ulcerations (diameter: > 2 cm)
ulcerative surface	none	< 10%	10–30%	> 30%
inflamed surface	unaffected segment	< 50%	50–75%	> 75%
presence of stenosis	none	singe, can be passed by endoscope	multiple, can be passed by endoscope	cannot be passed by endoscope

Table 5. Baron score and modified Baron Score

Score	Classic Baron Score	Modified Baron Score
0	Normal mucous membrane	Normal mucous membrane, vascular pattern visible, not friable
1	Abnormal mucous membrane, but without bleeding	Granular mucous membrane, vascular pattern not visible, not friable
2	Moderate bleeding – bleeding to light touch	1, but friable, no spontaneous bleeding seen
3	Severe bleeding – spontaneous bleeding	2, but spontaneous bleeding seen
4		3, but ulcerated, bare mucous membrane

In 2008 Gralinek et al. [38] created a capsule endoscopy scoring index, which is based on three endoscopic elements: villous edema, ulcers and stenosis, and integrates their number, range and additional descriptors. The score below 135 indicates normal mucous membrane or clinically insignificant mucosal inflammatory change. A mild condition is between 136 and 790 points and condition from moderate to severe is above 790 points.

Over the years, several indices for UC based on endoscopic imaging have also been established. In 1964 Baron et al. [39] proposed evaluating mucosal appearances in the colon and the rectum in UC by viewing the color, friability and moisture of mucous membrane, granularity, distensibility, presence of blood vessels, polyps, ulcers and blood in intestinal lumen. Based on the score, four stages of endoscopic activity were distinguished: 1) normal stadium with pale mucous membrane and visible vascular pattern, 2) inactive stadium, in which dry and granular mucous membrane is present, 3) moderately active stadium with moist, granular and friable mucosa, and 4) active stadium, in which mucous membrane is friable, moist and smooth (**Table 5**). A general endoscopic grading system, based on the stages described, has been established and has been used since with only a minor modification, introduced by Feagan et al. [21]. The Modified Baron Score (**Table 5**) allows the evaluation of whether the patient is in remission or not (score 1 or 0). While assessing lesions of colon mucosa, they distinguished a normal or inactive mucous membrane (0 points), mild changes, in which hyperemia, friability and fragmentary loss of vascular pattern occur (1 point), moderately active with massive hyperemia, erosions and completely loss of vascular pattern (2 point) and severe condition, in which spontaneous bleeding and ulceration occur (3 points). The Modified Baron Score is currently the most frequently used scale by clinicians [20] and until 2011 it has been the only validated endoscopic scale for measuring disease activity [40].

In 2012, the group of Travis et al. presented the Ulcerative Colitis Endoscopic Index of Severity (UCEIS), which included three variables: vascular pattern, bleeding and erosions and ulcers. Each variable is divided into three to four levels of severity with a comprehensive definition. This endoscopic scale, unlike previously developed indices, excluded friability of mucosa [41]. Since the first report, UCEIS has been validated and

showed to correlate well with overall assessment of severity [42]. Furthermore, UCEIS is now also viewed as a possible indicator for an early decision to use infliximab or ciclosporin (Corte et al., unpublished results reported at ECCO 2013).

The most recent scale, the Ulcerative Colitis Colonoscopy Index of Severity (UCCIS), was established by Neumann and Neurath in 2012 [42]. In this scale four parameters (mucosal lesions) are being assessed: vascular pattern, granularity, ulceration and friability (bleeding) (**Table 6**). Additionally, the index consists of four-point (0–3 points) grading of segmental assessment of endoscopic activity (SAES – assessing endoscopic severity of each colonic segment) and global assessment of endoscopic activity (GAES – assessing endoscopic severity of all five colonic segments). GAES is further shown as the 10 cm-visual analogue scale presenting severity of UC from normal to extremely severe. In 2013, Samuel et al. validated this endoscopic assessment tool [40].

Histopathological indices in IBD

The histological examination of endoscopic biopsies is a very useful tool to distinguish CD from UC. In 2013 the European Crohn's and Colitis Organization (ECCO) and the European Society of Pathology (ESP) published a Consensus containing recommendation for histopathological diagnosis of IBD [43], underlining its importance.

The histopathological assessment can be used to establish microscopic activity scales in CD and UC. The Riley index was created in 1991 [44]. In this four-point scale (0–3 points), six parameters are assessed: polymorphonuclear cells in lamina propria, chronic inflammatory cell infiltration, presence of crypt abscesses, mucin depletion, integrity of epithelial surface and irregular crypt structure.

In 1998, D'Haens et al. [45] developed the Scoring System for Histological Abnormalities in Crohn's Disease Mucosal Biopsy Specimens. In this scale 8 parameters were considered: epithelial damage, structure changes, infiltration of mononuclear cells in lamina propria, infiltration of polymorphonuclear cells in lamina propria, polymorphonuclear cells in epithelium, presence of erosion or ulcers, presence of granulomas and number of biopsy specimens occupied (**Table 7**).

The frequently used histopathological scale in UC, Geboes index, was created in 2000 [46]. The scale has 5 grades. Any architectural changes are indicated by grade 0. Grade 1 is characterized by the increase of chronic inflammation in the lamina propria. Increased level of eosinophils in the lamina propria is represented by grade 2A and increased of neutrophils – 2B. Grade 3 describes the presence of neutrophils in the epithelium. Expansion of crypt destruction is assigned to grade 4. Grade 5 indicates erosion and ulceration.

Table 6. Ulcerative Colitis Colonoscopic Index of Severity (UCCIS)

Variable	0	1	2	3	4
vascular pattern	normal vascular pattern	partially visible vascular pattern	complete loss of vascular pattern	–	–
granularity	normal, smooth and glistening	fine	leathery	–	–
ulceration	normal mucosa, lack of erosion or ulcer	presence of erosion or pinpoint ulcerations	presence of numerous, superficial ulcers covered by mucous	presence of deep, excavated ulcerations	widespread ulceration with >30% involvement
friability/bleeding	normal mucosa, no friability, no bleeding	friable, bleeding to light touch	spontaneous bleeding	–	–
grading of SAES and GAES	normal: visible vascular pattern and lack of bleeding, erosions, ulcers or friability	mild: presence of fine granularity and erythema, decreased or loss of vascular pattern, lack of mucous friability or spontaneous bleeding	moderate: presence of mucous friability and bleeding to light touch, coarse granularity, erosion or pinpoint ulceration	severe: presence of spontaneous bleeding or gross ulcers	–
GAES VAS 10cm scale	I - - - - - I - - - - - I - - - - - I - - - - - I - - - - - I - - - - - I - - - - - I - - - - - I - - - - - I - - - - - I Normal Extremely severe				

Table 7. The overview of the Scoring System for Histological Abnormalities in Crohn's Disease Mucosal Biopsy Specimens

Epithelial damage	0 – normal 1 – focal damage 2 – extensive damage
Architectural changes	0 – normal 1 – moderate lesions (< 50%) 2 – severe lesions (> 50%)
Infiltration of mononuclear cells in the lamina propria	0 – normal 1 – moderate infiltration (up to 2x the normal number of cells) 2 – severe infiltration (> 2x the normal number of cells)
Infiltration of polimorphonuclear cells in the lamina propria	0 – normal 1 – moderate infiltration (up to 2x the normal number of cells) 2 – severe infiltration (> 2x the normal number of cells)
Polimorphonuclear cells in epithelium	1 – in surface epithelium 2 – cryptitis 3 – crypt abscess
Presence of erosion and/or ulcers	0 – No 1 – Yes
Presence of granuloma	0 – No 1 – Yes
Number of biopsy specimens occupied	0 – none (0 of 6) 1 – ⩽ 33% (1–2 of 6) 2 – 33–66% (3–4 of 6) 3 – > 66% (5–6 of 6)

Conclusion and future perspectives

It has been more than a century since the very first descriptions of the CD and UC were reported and the research on IBD began. Currently, the numbers of IBD incidents are still increasing, not only due to higher morbidity, but also better diagnostic tools available. Following the progress in endoscopic and histopathological techniques, numerous indices and disease activity scales have been established for IBD. However, we still do not possess a perfect diagnostic tool or a disease marker or symptom, which would precede the development of the inflammation process for years and serve for disease indexing and facilitating IBD treatment. The indices nowadays are mainly used in clinical conditions to assess the response to drugs.

Clearly, the major problem nowadays is the lack of a single, internationally accepted and consistently used disease activity index for UC. Although numerous indices have been established, none is being used widely and/or properly; even the Mayo index, which is used in multiple clinical trials, exists in different forms, where the cutoff scores defining remission and response differ significantly. The use of several indices in different versions in clinical trials is only troubling the research processes and makes the data often hard to compare.

The index for clinical practice needs to be straightforward and easy to use. Ideally, one quantitative scale, with a short explanation of a score, would possibly facilitate the communication between physicians from completely different specialties. Another desired feature in clinical index is noninvasiveness, as repeated endoscopy can discourage potential clinical trial participants and is prone to inter-observer variation.

Importantly, none of the indices discussed above shows a significant prognostic value. Possibility to predict the clinical course of the disease over time comes with biological markers (for review see: [47]). The proposed number of potential markers is increasing, but we still lack the best. Ideally, the marker level should coincide with the process of mucosal healing, the latest target in IBD treatment. Of several possible disease markers, perinuclear antineutrophil cytoplasmic (pANCA) and anti-Saccharomyces cerevisiae antibodies (ASCA) seem to have the highest potential in being used on a larger scale. pANCA and ASCA have low sensitivity, but both were shown to distinguish UC from CD [48]. Fecal calprotectin (FC) and lactoferrin are also promising. FC with higher sensitivity and specificity than lactoferrin [49;50] appeared as a recommended marker used in clinical practice. The question whether these and other markers or symptoms will be widely used for IBD diagnosis and whether new disease indices are to be developed remains unanswered, but the need for an efficient IBD treatment requires prompt actions in this field.

New scanning techniques for IBD diagnosis and progress are emerging. Apart from refinement of endoscopy, which leads to procedures that are more efficient and safer for patient, methods like magnetic resonance enterography have been evaluated and initially approved to be used in clinical conditions, e.g. for patient inclusion to therapeutic trials (Higgins et al., unpublished results).

A better understanding of IBD causes, standardization of definitions and validation of new disease indices, evaluation of long-term effectiveness of drug therapies and many more may now be assured – among others – by two groups established in the early 1980's, International Organization For the Study of Inflammatory Bowel Disease (IOIBD) and Groupe d'Étude Thérapeutique des Affections Inflammatoires du Tube Digestif (GETAID). The initial aim of IOIBD was to agree on an international index for CD activity, which further led to splitting the discussion into for topics: the clinical features of severity, a morphological index based on radiological/endoscopic features, an index based on laboratory parameters of inflammation and a nutritional index and assigning task forces related to epidemiology, clinical trials or surgery. Recent activity of IOIBD continues to address the initial goals like validation of endoscopic activity scores [52], but has expanded to finding new IBD biomarkers [53] or long-term therapeutic and side-effects of anti-IBD drugs [54]. The ongoing projects of GETAID include, inter alia, the FER study, which seeks for effective treatment of CD patients suffering from anemia, TAILORIX, which evaluates the benefits of the anti- IBD treatment with infliximab, or MICA, whose aim is to characterize the effect of adalimumab in IBD patients with intra-abdominal or pelvic abscess. They are mostly multi-center studies, involving large patient cohorts and with both, clinical and educational aspects.

To conclude, it has been more than a century since the first case of IBD was reported. However, a massive progress in the studies on the disease was made recently, which warrants many new exciting developments in the field of epidemiology, therapy and healthcare organization with regard to IBD in the very near future. They will shape the view of the clinical aspects of the disease and are expected to facilitate its better diagnosis and treatment.

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Conflict of interest statement

The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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Predicting the risk of atherosclerosis in patients with cystic fibrosis – rationale and design of a prospective cohort study

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ABSTRACT

The project "Risk of atherosclerosis in cystic fibrosis in relation to the exogenous and endogenous factors that influence the course of the disease" ranked first in the OPUS2 Competition, as announced in May 2012 by the Polish National Science Center. The total value of the grant is 198,580 PLN (ca. 50,000 EUR). The grant was awarded jointly to the Department of Pediatric Gastroenterology and Metabolic Diseases and to the Department of Cardiac Intensive Care at Poznan University of Medical Sciences, Poland. The project will be focused on conducting a prospective cohort study in patients with cystic fibrosis (CF) and healthy controls. Cases of symptomatic and asymptomatic forms of coronary heart disease in patients with CF were reported [1, 2]; however, no data on the epidemiology of atherosclerosis in patients with CF were published so far. In the past, cardiovascular disease in patients with CF used to be limited to pulmonary heart disease as a consequence of end-stage chronic obstructive pulmonary disease [3]. Although hypertension has not yet been officially recognized as a major problem in this population [4], there are reports indicating that it is found in 20% of patients in adult CF care centers [5]. The project is innovative in nature and necessitates close co-operation between cardiology and basic science units.

Keywords: atherosclerosis, cystic fibrosis, coronary heart disease.

General information

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The project will be focused on conducting a prospective cohort study in patients with cystic fibrosis (CF) and healthy controls. Cases of symptomatic and asymptomatic forms of coronary heart disease in patients with CF were reported [1, 2]; however, no data on the epidemiology of atherosclerosis in patients with CF were published so far. In the past, cardiovascular disease in patients with CF used to be limited to pulmonary heart disease as a consequence of end-stage chronic obstructive pulmonary disease [3]. Although hypertension has not yet been officially recognized as a major problem in this population [4], there are reports indicating that it is found in 20% of patients in adult CF care centers [5].

The project is innovative in nature and necessitates close co-operation between cardiology and basic science units.

The Basic Concept of the Research

Epidemiological data indicate that risk factors for cardiovascular disease observed among children, adolescents and young adults have a prognostic significance for adulthood [6]. CF guidelines that can be found in the January 2004 Consensus Report were designed to help with the transition of CF health care from pediatricians to internists or other adult care providers. Many aspects of cardiovascular disease other than pulmonary heart disease, e.g. hypertension, are missing from this consensus report [7]. There is a need to continually update and extend our knowledge of aging-related diseases in CF patients [8]. The fact that cases of asymptomatic and symptomatic coronary artery disease have been reported in association with CF suggests that life expectancy in adult CF patients has reached sufficient length so as to make artery disease a concern. This also illustrates the need to perform systematic research in this area [9, 10].

Research Project Objectives

The purpose of the project is to evaluate the risk of atherosclerosis in CF patients in relation to the exogenous and endogenous factors that influence the course of the disease. Early (subclinical) risk factors of atherosclerosis will be evaluated. In addition, the academic significance of the project is strengthened by its potential to identify the relationship between atherosclerotic changes and genetics, as well as organ-specific and systemic CF-related pathology.

Research Methodology

Study Population

The study will enroll patients with CF. The control group will consist of 50 healthy volunteers matched to CF patients according to gender and age. The written consent to participate in the study will be obtained from all entrants and, in cases of underage study participants, also from their parents. The summary of inclusion and exclusion criteria is shown in **Table 1**.

Methods

In all examined patients the following parameters characterizing the clinical expression of disease will be assessed:

1. Nutritional status – using typical anthropometric parameters: standardized body height and weight (Z-score), standardized body mass index (BMI Z-score), and a biochemical parameter – serum albumin concentration;
2. Diet – using a 7-day consumption questionnaire and calculations performed using "Dietician" software;
3. Lung function – using spirometry (FEV1 – forced expiratory volume in 1 second – expressed as a percentage of predicted value);
4. Respiratory tract colonization by Pseudomonas aeruginosa – based on the results of sputum culture;
5. Exocrine pancreatic function – based on the measurement of elastase-1 concentration in feces;
6. Parenchymal liver damage and cholestasis (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase).

   In addition, detailed information concerning:
   - the supplementation of pancreatic enzymes,

Table 1. Inclusion and exclusion criteria for patients with cystic fibrosis (CF) and healthy subjects

Cystic fibrosis group	Control group
Inclusion criteria: 1. CF diagnosed based on medical history confirmed by the molecular analysis of mutations in the CFTR gene, clinical picture, elevated concentrations of chloride anions in sweat 2. Age >16 years 3. Consent to participate in the study.	Inclusion criteria: 1. Age >16 years 2. Consent to participate in the study.
Exclusion criteria: 1. Positive family history of dyslipidemia (hypercholesterolemia, hypertriglyceridemia), 2. Early episodes of coronary artery disease and cerebrovascular diseases in the family (occurrence of episodes of the disease before the age of 65 years in women and before 55 years in men).	Exclusion criteria: 1. Positive family history of dyslipidemia (hypercholesterolemia, hypertriglyceridemia), 2. Early episodes of coronary artery disease and cerebrovascular diseases in the family (occurrence of episodes of the disease before the age of 65 years in women and before 55 years in men).

- inhaled or oral antibiotics taken permanently,
- the last oral and intravenous antibiotic-therapy will be collected.

In all study participants (CF and control groups) the following parameters related to the prediction of the risk of atherosclerosis will be assessed using enzyme-linked immunosorbent assay (ELISA): adhesion proteins (sP-selectin, vascular cell adhesion molecule-l), asymmetric dimethylarginine, apolipoprotein E, adiponectin, oxidized low-density lipoprotein, high sensitivity C-reactive protein. Apolipoproteins A-1 and B will be estimated using the turbidimetric method. The lipid profile (total cholesterol, triglycerides, high density lipoproteins and low-density lipoproteins) will be assessed using the enzymatic colorimetric method.

The assessment of vascular changes will involve a non-invasive measurement of carotid intima media thickness (CIMT) in all the study participants (using high-resolution ultrasound system equipped with a linear head) and an analysis of vascular stiffness (50 CF patients; 30 healthy volunteers). The latter will be evaluated on the basis of two parameters: the speed of pulse wave assessed using photoplethysmography and the analysis of pressure waveforms and central hemodynamics using commercial equipment.

Expected Results

The project will improve the understanding of the course of atherogenesis in patients with CF, a topic on which no specific studies were published so far. It is expected that the results will shed light on the role of both exogenous and endogenous determinants of atherosclerosis in patients with CF. The study will provide new information regarding the atherosclerotic process in nonobese individuals, who have often been neglected in the studies of atherosclerosis as a low risk group. In the future, the newly obtained insight might translate to improved medical care in CF patients.

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Conflict of interest statement

The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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Project "Equalizing opportunities – raising competence of children with disabilities"

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ABSTRACT

The project will enable the use of the Tomatis therapy for children with central auditory processing disorders and the introduction of a proprietary curriculum preparing children to learn English in primary school – based on the method that streamlines the auditory analysis, on the proprietary language integration CDs used in the Tomatis method and on individual educational and therapeutic lessons with the teacher. The curriculum will be developed in the course of research under the guidance of prof. UM dr hab. Ewa Mojs from the Poznan University of Medical Sciences. The leader of the consortium implementing the project is the Foundation for Local Activity. The innovation has a high potential for implementation. Applicants to begin with estimate ultimately the ability to implement the program in the first year in 350 schools with the right equipment.

Keywords: Tomatis therapy for children, primary school.

General information

Project "Equalizing opportunities – raising competence of children with disabilities" was awarded a grant from The National Centre for Research and Development (Narodowe Centrum Badań i Rozwoju, NCBR) (grant number: IS-2/24). The project is run by a consortium that includes:

- Fundacja Aktywności Lokalnej (Foundation for Local Activity) – Consortium leader,
- Learn Up Sp. z o.o.,
- Poznan University of Medical Sciences.

The main project team consists of: prof. UM dr hab. Ewa Mojs (Project Manager), prof. dr hab. Włodzimierz Samborski and mgr Beata Marciniak.

Ethics

Bioethical Committee of Poznan University of Medical Sciences on 8th November, 2012 gave its approval for the project (number 882/10).

Project objectives and concept

The goal of the project is to develop and implement an innovative educational program that will help to prepare primary school pupils to learn English. The educational program is based on Tomatis Method – a method of sound sensory stimulation, authorial language recordings that are used in the Tomatis Method, and individual educational-therapeutic sessions. The program will be implemented in primary schools in Poland.

The Tomatis Method was created by Alfred Tomatis, a French otolaryngologist in the fifties of XX century [1, 2]. Alfred Tomatis was studying the relationship between the ear and the voice, and in extension, between listening and communication. His discoveries were summarised in three laws that explain the influence of listening and voice emission on behaviour and competences [2, 3]:

- the voice contains only these frequencies that ear can hear (the ear and the larynx are parts of the same neuronal loop),

– if hearing is modified, the voice is immediately and unconsciously modified,
– auditory stimulation undertaken over a certain time may result in permanent phonation transformation.

Tomatis Method is based on a distinction between hearing and listening [3, 4]. Hearing is a passive process of sound perception, that doesn't require conscious action. Listening, on the other hand, is a very active process, it consists of intentions, willingness and conscious focusing of attention. Listening is an ability that can be disrupted, disordered or lost, but also it can be regained. Listening deficits can be caused by medical and social factors, such as ear infections, head injuries, severe stress or trauma. Tomatis Method (via authorship rehabilitation device called the electronic ear) through direct influence on the middle ear adapts its efficiency which allows to open a specific frequency band that is optimal for information processing. Tomatis Method is used in rehabilitation of patients with such disorders as: ADHD, ADD, dysgraphia, dyslexia, autism and Asperger syndrome, stuttering or speech and language delay [4].

The project's main focus is on helping Polish primary school pupils to master English skills. Poland experiences problem with low level of foreign language skills in children and teenagers. This is especially important in regard to children with specific learning needs. According to European Survey on Language Competences (ESCL) from 2011 Polish pupils have one of the lowest levels of language competences in Europe. The project's goal is to develop an educational-therapeutic program that will help pupils in learning English by utilizing therapeutic effects of Tomatis Method.

Project plan

Task 1. Building and defining of research area

The goals of this task are: to gather and summarise up to date knowledge about modern methods of cognitive rehabilitation in children; performing preliminary quantitative study of 80 children (aged 6–11), 40 of which will have diagnosis of dyslexia. The study results will help to define and specify the research area. The initial level of language and other psychological skills of the research group will be determine.

Task 2. Test study

The main study of 80 children (aged 6–11) with normal intelligence level and auditory processing deficits. A subgroup of 40 children will be also diagnosed with dyslexia. Clinical psychologist will examine children 4-months after implementation of educational-therapeutic program based on Tomatis Method (n = 80), and after simultaneous implementation of individual educational-therapeutic English sessions (only for 40 randomly selected participants).

Task 3. Final test study and development of application for school purposes

The goal is to evaluate the long term effectiveness of the educational-therapeutic program. The final psychological evaluations will be performed one year after the implementation of the program in the research group. This task consist also of a control group (n = 80) evaluation and its comparison to research group in the field of language skills. After the evaluation an application that can be used in terms of mass schools will be developed.

Task 4. Measures preparing for implementation of the program in schools

The project expects that the program may be implemented in 350 primary schools in Poland that already have the necessary equipment for Tomatis Method. To do so it is necessary to develop tools for teachers that will use this method. A vital part of this task will be creating an e-learning program, open to all interested, that will allow to explore the method and get to know how it may be used in teaching English.

Expected results

The project has high scientific value and it's a continuation of previous research made on Tomatis Method in Poznan University of Medical Sciences. The research results are expected to be implemented within one year from completion of the project. The project expects that the program should be fairly easily implemented in about 350 primary schools in Poland – the ones that already have the necessary equipment for Tomatis Method.

The project provides adequate solutions to target group's problems. The educational-therapeutic program can be especially beneficial for children with auditory processing deficits – specifically for children participating in the research, but also in general population. It will help in learning foreign languages, a skill that is extremely important nowadays. The project's impact will be nationwide. Its direct users will be schools and teachers, and its beneficiaries will be children with specific educational needs.

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Acknowledgements

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All manuscripts should use the 'Vancouver' style for references. References should be numbered consecutively in the order in which they appear in the text **and listed at the end of the paper.** References cited only in Figures/Tables should be listed in the end. Reference citations in the text should be identified by Arabic numbers in square brackets. Some examples:

This result was later contradicted by Smith and Murray [3].
Smith [8] has argued that...
Multiple clinical trials [4–6, 9] show...

List all authors if there are six or fewer; if there are seven or more, list first six follower by "et al.". Journal names should be abbreviated according to Index Medicus.

Some examples

Standard journal articles

1. Fassone E, Rahman S. Complex I deficiency: clinical features, biochemistry and molecular genetics. J Med Genet. 2012 Sep;49(9):578–590.

2. Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D et al. The genetic landscape of high-risk neuroblastoma. Nat Genet. 2013 Mar;45(3):279–284.

Books

Personal author(s)

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

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