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ORIGINAL PAPER

Katarzyna Pogoda [1,2(ABCDEFG)], Paweł Rybojad [3(BFH)], Inés Ramírez Álvarez [4(B)], Thusyanthan Mohanathas [4(B)], Sarah Hosseini [4(B)], Jacek Tabarkiewicz [1,2(ABCDEFH)]

Optimization of detection of circulating tumor cells by flow cytometry and qRT-PCR

[1] Department of Human Immunology, Institute for Experimental and Clinical Medicine, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[2] Centre for Innovative Research in Medical and Natural Sciences, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[3] Department of Thoracic Surgery, 2nd Faculty of Medicine, Medical University of Lublin, Lublin, Poland
[4] Students' Scientific Association at Department of Clinical Immunology, 2nd Faculty of Medicine, Medical University of Lublin, Lublin, Poland

ABSTRACT

Introduction. Treatment and diagnostic process in solid tumors like lung cancer are still based on invasive methods such as bronchoscopy, solid biopsy et cetera. One of the less invasive methods is a proposed "liquid biopsy" which is based on capturing of tumor cells circulating in the blood.

Aim. The aim of the study was to standardize conditions and to assess the sensitivity of the identification of circulating tumor cells (CTCs) with the use of flow cytometry and qRT-PCR.

Material and methods. In the first model of CTCs, cells from the A549 lung cancer cell line were suspended in 1 ml of healthy donors' blood in 5 spikes increasingly: 0, 10, 50, 100 and 200 and the cells were detected in flow cytometer. In the second model, cells from the A549 and H1975 lung cancer cell lines were used. Spikes were prepared as in the first model, but cells were suspended in 400 µl of healthy donors' blood and were detected with the use of qRT-PCR.

Results. An increasing number of detected cytokeratin positive events from the 1st spike "0" to the last one - "200" was observed by flow cytometry. Median value in the negative control was 0 false positive cells. In tubes from "10" to "200" the median was 5, 43.5, 58 and 78, respectively. Mean sensitivity of flow cytometry was 63.79%. In qRT-PCR, correlation between increasing number of sorted cells in several spikes and the level of mRNA expression for KRT19 gene was not observed.

Conclusion. Commonly available methods like flow cytometry and qRT-PCR seem to be attractive solutions for CTCs detection, but they need pre-enrichment procedures and standardization.

Keywords. circulating tumor cells, flow cytometry, PCR

Corresponding author: Katarzyna Pogoda, e-mail: kpogoda@ur.edu.pl

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

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Introduction

Circulating tumor cells – liquid biopsy

Circulating tumor cells (CTCs) were observed for the first time by Thomas Ashworth, a pathologist from Australia, in 1869. He identified cells similar to cancer cells during an analysis of a postmortem blood sample.[1] Subsequently, in 1954, Watanabe observed that clusters of tumor cells injected into murine circulatory systems have great metastatic potential.[2] From this time on, we obtained wide knowledge about properties and functions of circulating tumor cells. A sparse population of circulating tumor cells has been estimated in the circulatory system of patients with advanced cancer at one CTC per billion normal blood cells.[3] CTCs are bigger than white and red blood cells; they have a diameter of ~ 12-25 μm vs 7-15 μm and 8 μm diameter for WBCs and RBCs, respectively.[4] Today, we know that this sparse cell population could be found in the blood because tumor cells are able to migrate into the bloodstream. They spread in whole circulatory system by angiogenesis promotion and intravasation. Tumor cells produce some factors, like vascular endothelial growth factor (VEGF), IL-8, TNF, which stimulate neovascularization; endothelial cells promote the growth of tumor cells. CTCs are able to adhere to endothelium and to form metastasis in distal organs.[5]

CTCs can occur as a single cell and also as a clustered (CTM – circulating tumor microemboli) group of more than three tumor cells that travel together in the bloodstream. The life span of CTM in the bloodstream is shorter than a single cell, which is able to exist in the circulatory system for only several hours. However, it was shown that clusters have a greater predisposition of forming distal metastases than single tumor cells. CTM can be derived from a primary tumor and also can be formed by aggregation or proliferation of single CTCs. They were identified in several cancers such as lung, breast, colorectal, prostate cancer, and also in melanoma or glioblastoma.[6]

During investigation of CTCs phenotype, there was increasing evidence that they are a highly heterogeneous population. At the beginning, they were described as CD45-/EpCAM+/CK+ cells. However, today we know that CTCs partially undergo epithelial-to-mesenchymal transition (EMT). Moreover, part of them are described as circulating tumor stem cells and they cannot be detected with methods based only on epithelial markers.[7] Because EMT+ CTCs were identified in cancer patients, who were classified as CTCs negative with the use of the EpCAM based method, scientists are still trying to find proper markers to catch CTCs.

Why are researchers trying to detect, isolate and analyze circulating tumor cells? What do we want to know from such a sparse population? The main reason for interest in CTCs is the idea of a non-invasive cancer diagnostic strategy named "liquid biopsy".[8] It is based on the assumption that it would be possible to diagnose, set a treatment strategy, and monitor the patient using only a few milliliters of whole blood instead of traditional imaging and solid biopsy. This idea has been developed and the Food and Drug Administration has approved clinical use of the CellSearch System for CTCs isolation in metastatic breast, colorectal and prostate cancer.[9] However, because of the heterogeneity of CTCs, proper isolation of this population is still unavailable, because we do not have a technique which would be able to identify EpCAM+ CTCs, CTCs undergoing EMT process, circulating tumor stem cells, and CTCs clusters simultaneously.

Techniques of CTCs detection

Today, the capturing of CTCs is a widely explored issue. Reviews describing development of methods created for CTCs detection mention dozens of techniques.[3,10–13] They are based on immunoaffinity or biophysical properties of tumor cells. The first aforementioned technology is used in two forms – as positive or negative enrichment.

Positive enrichment means that cells are captured only if they show expression of several surface markers. Positive enrichment is used in many devices designed for CTCs detection, eg. CellSearch System, AdnaTest, MACS, MagSweeper, Isoflux or GILUPI CellCollector.[10] The main surface marker that positive enrichment is based on is EpCAM. After capturing CTCs with the anti-EpCAM antibody, evaluated cytokeratin (CK) expression is evaluated and DAPI staining is prepared. Prognostic value of EpCAM was approved during validation of the CellSearch system in breast and prostate cancer. However, as mentioned above, CTCs are a heterogeneous population and by using EpCAM, we can identify only epithelial cells, and we lose CTCs undergoing EMT and the others.[14]

Although, there is another branch of techniques of CTCs detection based on immunoaffinity termed negative enrichment. CTCs population is obtained by depletion of CD45 positive cells. Therefore, we can detect all types of CTCs, but we do not obtain such a pure population as in the positive enrichment technique. Here we employ, for instance, the EasySep Human CD45 Depletion Kit or Negative Enrichment Immunofluorescence and an In Situ Hybridization System.[15,16]

Methods based on the biophysical properties of tumor cells are very different. Here we have techniques based on size and deformability of tumor cells, techniques using density gradient centrifugation (RosetteSep-CTC, Accucyte Enrichment and CyteSealer), microfiltration in two (ISET, FMSA) and three dimensions (Resetteable Cell Trap, Cluster Chip), inertial focusing (Vortex, ClearCell FX), electrophoresis (DEPArray) or acoustophoresis (Acoustophoresis Chip).[10]

Most of the methods mentioned require special technical devices which causes CTCs isolation to be quite expensive. The costs and problems with standardization are the main reasons that CTCs cannot be commonly detected in a routine diagnostic process for monitoring cancer patients. Therefore, we had the idea to verify the usefulness of commonly available laboratory techniques, such as flow cytometry (FC) and qRT-PCR, for CTCs identification.

Aim

The aim of the study was to standardize the conditions of CTCs identification with the use of FC and qRT-PCR. Sensitivity of FC and qRT-PCR were assessed in 2 models prepared from lung cancer cell lines suspended in healthy donors' whole blood.

Material and methods

Flow cytometry

Cells from the A549 lung cancer cell line (American Type Culture Collection, Manassas, VA, USA) were cultured under standard conditions: 37°C, 5% CO2 and 95% humidity in a culture medium that consisted of Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, Saint Louis, MO, USA) and Dulbecco's Modified Eagle's Medium_Nutrient Mixture F-12 Ham (Sigma-Aldrich, Saint Louis, MO, USA) with addition of Fetal Bovine Serum (Biochrom GmbH, Berlin, Germany) and Penicillin-Streptomycin-Neomycin Solution Stabilized (Sigma-Aldrich, Saint Louis, MI, USA). 5 ml peripheral blood samples were taken from 6 healthy volunteers into heparinized tubes. The first portion of peripheral blood from each donor was rejected to avoid sample contamination with epithelial cells. Subsequently, the blood sample was divided into 5 cytometric tubes (1.0 ml per tube) and then 5 different suspensions were prepared (0 cancer cells/ml, 10/ml, 50/ml, 100/ml and 200/ml) of cancer cells from A549 lung cancer cell line in healthy donor blood with the use of an electronic pipette (Eppendorf, Hamburg, Germany). Before preparation of the spikes, cancer cells had been counted with a Countess Automated Cell Counter (ThermoFisher Scientific, Waltham, MA, USA).

The next part of the experiment consisted of intrinsic molecule labeling with monoclonal antibodies (mAbs) and sample acquisition with the use of BD FACSCanto II Flow Cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Mouse anti-human pancytokeratin FITC mAb was used (Miltenyi Biotec, Bergisch Gladbach, Germany). We did not identify expression of EpCAM on the surface of our cancer cell line, which is why we used only pancytokeratin mAb. After 20 minutes of incubation with mAb in the dark, each sample was incubated for 10 min with 3 ml of BD FACS Lysing Solution (Becton Dickinson, Franklin Lakes, NJ, USA) diluted in deionized water (1:9) at room temperature in the dark. Subsequently, samples were centrifuged for 5 min with 500 × g acceleration and washed in Phosphate Buffered Saline w/o Ca^{2+}, Mg^{2+} (PAA Laboratories GmbH, Pasching, Austria). In the last step samples were acquired. It was important to minimize the Electronic Abort Rate to avoid losing epithelial cells, so samples were acquired with the lowest available velocity. In consequence, average time of acquisition for one sample was 3 hours.

Real Time qRT-PCR

In the Real Time qRT-PCR experiment, we used two lung cancer cell lines – the first one was A549 as in the flow cytometry experiment and the second one was H1975 (American Type Culture Collection, Manassas, VA, USA). They were cultured under standard conditions. The H1975 cell line was cultured in RPMI 1640 Medium (PAA Laboratories GmbH, Pasching, Austria) with Fetal Bovine Serum (Biochrom GmbH, Berlin, Germany) and Penicillin-Streptomycin-Neomycin Solution Stabilized (Sigma-Aldrich, Saint Louis, MI, USA). After digestion with the use of Accutase Cell Detachment Solution (Corning, NY, USA) cancer cells were labelled with mouse anti-human EpCAM APC mAb (Miltenyi Biotec, Bergisch Gladbach, Germany) and mouse anti-human pancytokeratin FITC mAb (Miltenyi Biotec, Bergisch Gladbach, Germany). In the last step, spikes of cancer cells in healthy donor blood were prepared.

4 ml peripheral blood samples were taken from 6 healthy volunteers and placed into tubes with EDTA. The first portion of blood from each donor, as in the flow cytomeric model, was used for another procedure, because we wanted to avoid sample contamination with epithelial cells from the injection. Subsequently, the blood sample was divided into 5 eppendorf tubes (400 µl per tube) and then 5 different suspensions (0 cancer cells/0.4 ml, 10/0.4 ml, 50/0.4 ml, 100/0.4 ml and 200/0.4 ml) of cancer cells from A549 and H1975 lung cancer cell lines in healthy donor blood with the use of BD FACSAria III Cell Sorter were prepared. The population that we focused on was gated according to the scheme presented in Fig. 1.

In this model, cancer cells were sorted to 0.4 ml of blood because this is the volume dedicated for the MagCore HF16 Plus Automated Nucleic Acid Extractor (RBC Bioscience Corporation, New Taipei City, Taiwan) that was used for RNA isolation. The procedure of isolation was prepared according to the manufacturer's protocol with the use of kit number 601. RNA purification was prepared in an automated extractor to standardize the method. Subsequent steps were reverse transcription with the use of High Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, Waltham,

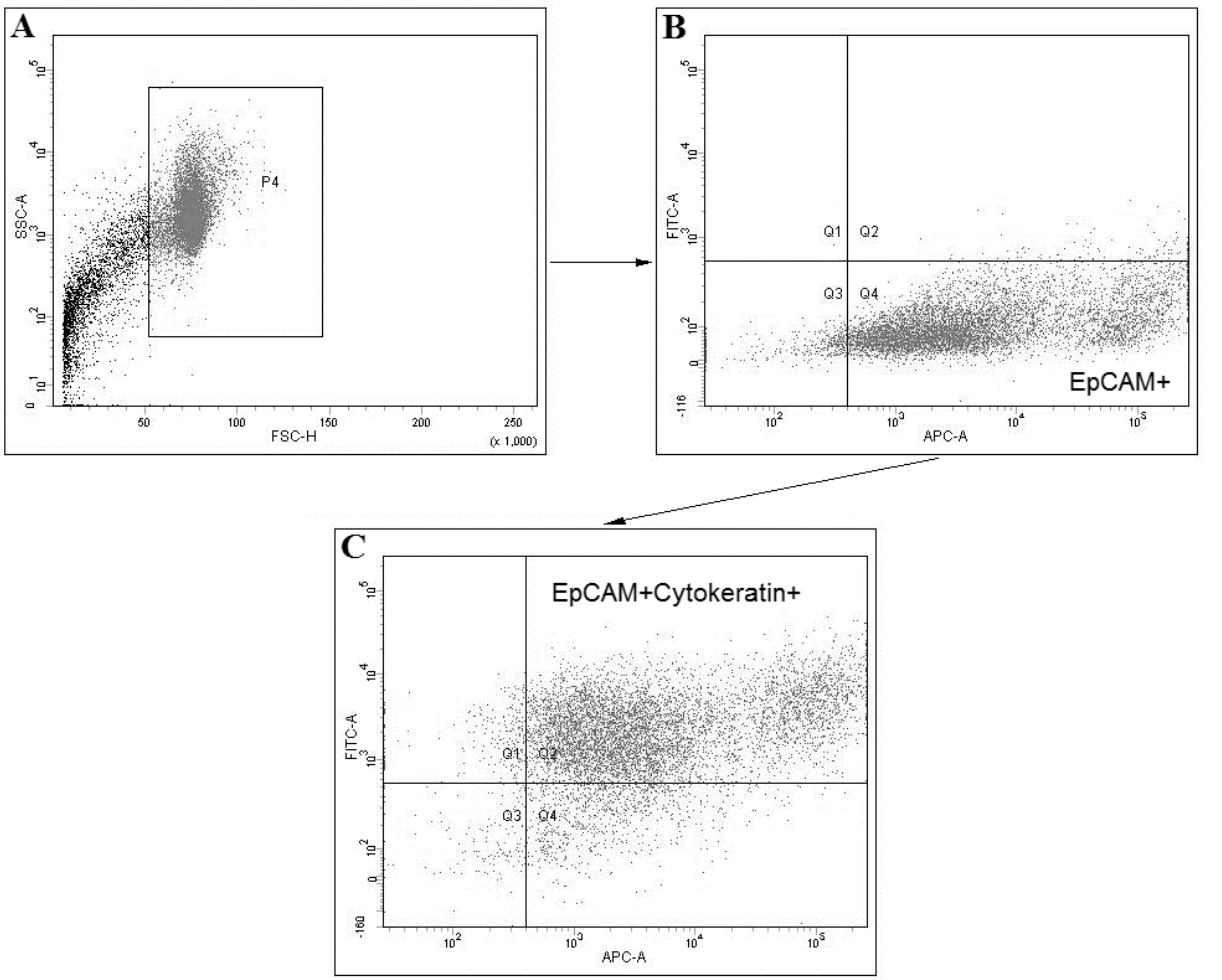

Fig. 1. Before sorting it is necessary to identify proper cell population. The scheme of gating lung cancer cell line cells in FC which were subsequently sorted to several spikes. A. P4 – H1975 lung cancer cell line population; B. Q4 – EpCAM positive H1975 lung cancer cell line population (extracellular staining); C. Q2 – EpCAM and pancytokeratin positive H1975 lung cancer cell line population (intracellular staining)

MA, USA) and the assessment of mRNA expression for KRT19 gene with the use of TaqMan Gene Expression Assay (ThermoFisher Scientific, Waltham, MA, USA). As a housekeeping gene control, we used TaqMan GAPDH Control Reagents (human) (ThermoFisher Scientific, Waltham, MA, USA). We pooled cDNA from '0' samples and we used ΔCt from samples '0' as a calibrator for normalization of qRT-PCR results. qRT-PCR was conducted with the use of Light Cycler 480 II Instrument (Roche, Basel, Switzerland).

Statistical analysis

Obtained flow cytometric and Real Time qRT-PCR data were collected in Microsoft Excel (Microsoft, Redmond, WA, USA) and analyzed by Statistica 13.0 PL software (StatSoft Polska, Cracov, Poland). The ANOVA Friedman test was used to verify the differences between the number of cells detected in several spikes. The significance of the differences between the predicted and detected cell number was assessed with the use of the Wilcoxon test. The percentage of samples with a lower number of detected CK+ cells than predicted in several spikes was calculated in frequency tables. We calculated correlation ratios (R2) and we described sensitivity of FC and Real Time qRT-PCR.

Results

Flow cytometry

Using FC, we observed an increasing number of detected CK+ events from the first spike "0" to the last one - "200". The number of events acquired in each sample was 3×10^6. In the negative control, the median value was 0 false positive cells. In the subsequent four tubes from "10" to "200", the median was 5, 43.5, 58 and 78 number of events, respectively. The coefficient of correlation between spiked cell number and detected cell number was R2 = 0.8795 (Fig. 2). Flow cytometry was characterized by 66.67%, 76.33%, 60.33% and 51.83% sensitivity in groups 10 to 200, respectively. Mean sensitivity was 63.79%.

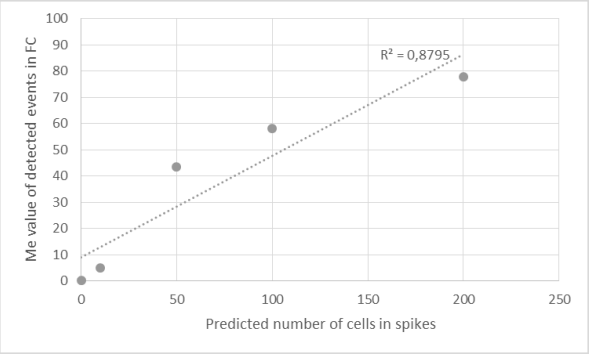

Fig. 2. The coefficient of correlation (R2) between spiked cell number and detected cell number in flow cytometry

According to the Wilcoxon test, there were not statistically significant differences between predicted and the real number of CK+ events in any group. However, dispersion in all spikes was very high (Table 1).

Table 1. A comparison between predicted and real numbers of CK+ events detected by flow cytometry

	Tube "10" [Me]	Tube "50" [Me]	Tube "100" [Me]	Tube "200" [Me]
Predicted number of cells	10	50	100	200
Detected number of cells	5	43.5	58	78

According to frequency tables, in 66.67% of spikes "10", fewer events were detected than predicted. In spikes "50" fewer events than predicted were detected in 50.00% of samples. Spikes "100" and "200" gave 62.50% and 75.00% fewer events than detected respectively.

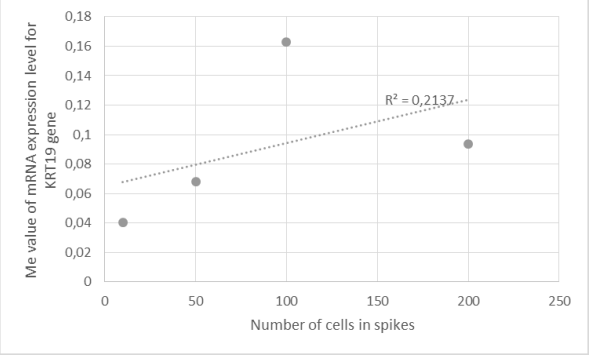

Fig. 3. Coefficient of correlation (R2) between spiked cell number and the level of mRNA expression for KRT19 gene in Real Time qRT-PCR

Real Time qRT-PCR

With the use of Real Time qRT-PCR, we were able to detect expression of mRNA for KRT19, but we did not observe a correlation between the increasing number of sorted cells in several spikes and the level of mRNA expression. There assumed increase of mRNA expression from the "10" to "200" spike in the model prepared with the use of the A549 cell line was not observed. The model with the H1975 cell line showed an increasing tendency in mRNA level, but the correlation ratio between the number of cells and mRNA level was very low (R2 = 0.2137) (Fig. 3).

Discussion

Flow cytometry, a conventional method available as standard equipment in many medical facilities, may be an attractive way for detection of CTCs according to Takao et al.[17] Rapid readout of routine measurements, the capability of multicolor analysis, and the fact that size information is included in the data are the main advantages of this method. The same results were reported by Lu et al.[18] With the previous magnetic depletion of CD45+ cells, they detected CTCs with 87.5% sensitivity. Our FC results were characterized by 63.79% mean sensitivity. However, an important fact is that the mentioned research that focused on CTCs detection with FC were equipped with additional devices for enriching the population prior to using FACS. In our experiment, we wanted to use FC without any supplementary devices to avoid generation of additional costs. Nevertheless, many other techniques for CTCs detection also require pre-enrichment.[13,19] Therefore, it is possible that even if this step generates additional costs and cell loss, pre-enrichment is necessary to obtain an acceptable method sensitivity.

qRT-PCR is very often used in CTCs detection techniques only for molecular characterization of isolated populations.[20-22] Fu et al. assessed in CTCs expression of hTERT mRNA level, while Bao et al. prepared multimarker qRT-PCR and assessed mRNA expression for 8 genes.[20,22] In some papers, qRT-PCR was used for CTCs identification, but only after pre-enrichment with the use of MACS or another negative enrichment techniques.[21,23,24] According to our experiment, where we isolated mRNA directly from whole blood, the correlation between the number of spiked cancer cells and expression of mRNA for KRT19 was weak (R2 = 0.2137). One of the problems with using qRT-PCR for CTCs detection is the lack of a standardized set of markers characterizing this population. Koren et al. prepared a similar experiment for CTCs detection with the use of qRT-PCR and obtained strong positive linear relationship between the number of spiked cells and the level of mRNA expression (R2 = 0.998), but they assessed the expression of mRNA for KRT7.[25] On the other hand, there was a study which compared qRT-PCR with the CellSearch System and in this experiment Politaki et al. assessed the level of KRT19 mRNA expression and obtained concordant results in qRT-PCR and in the CellSearch platform in samples from patients with metastases breast cancer.[26]

Although, they took 20 ml of blood from patient and isolated mRNA from PBMC.

The main assumption of our study was that the methodology should be simple, but it was connected with many limitations. The most critical point in the study was preparation of CTCs models in whole blood of healthy donors. We have to consider the influence of pipetting errors and dilutions during preparation of the spikes. Cancer cells were obtained from cell culture which were scrapped or enzymatically digested, suspended in PBS and counted. Because of the large number of cells, suspensions needed to be diluted. All these steps are a source of error, which Koren et al. eliminated by preparation of the spikes with the use of micromanipulator system.[25] Also, during acquisition in FC, we lost part of events and similarly during centrifugation in procedure of intrinsic molecules staining. The situation most often occuring in FC was that the number of detected positive events was underestimated. A high number of false negative events in FC is associated with the risk of omitting CTCs. On the other hand, in several samples, we detected also false positive events in FC. This could be associated with low precision during preparation of the spikes.

Different CTCs detection assays are created that vary considerably in the protocols and markers used for CTCs isolation, the volume of blood analyzed as well as the definition of positivity. Therefore, comparisons between experiments are difficult. As a consequence, despite the fact that in multiple reports the presence of CTCs has been correlated with patient outcome, CTCs have not as yet been approved for prevalent clinical use.

Some groups of researchers are focused on detection of CTCs in lung cancer patients to replace invasive diagnostic procedures with 'liquid biopsy'.[27-29] Nonetheless, the gold standards today are still chest X-ray, bronchoscopy and solid biopsy.

Conclusion

To conclude, we have many methods to identify circulating tumor cells in use today. However, if they are to become useful for clinicians, they need to be standardized and simplified. For proper evaluation of FC and qRT-PCR usefulness in CTCs detection, highly precise equipment seems to be essential. Such commonly available and flexible methods seem to be a more attractive tool in diagnostic procedures than special devices created for CTCs detection. However, a set of the most characteristic markers for CTCs needs to be defined.

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ORIGINAL PAPER

Anna Pękala [1(ABG)], David Aebisher [2(DEFG)], Piotr Pardak [1,2(BFG)], Rafał Filip [1,2(ABDFG)]

Efficacy and safety of biosimilar IFX (CT-P13) and adalimumab in patients with active fistulizing perianal Crohn's disease naïve to anti-TNF therapy: preliminary results from the POLIBD study

[1] Department of Gastroenterology with IBD Unit, Clinical Hospital No. 2, Rzeszów, Poland
[2] Faculty of Medicine, University of Rzeszów, Rzeszów, Poland

ABSTRACT

Introduction. The development of perianal fistulas are a risk factor in colonic and rectal disease. Perianal CD treatment requires a combination of surgical and therapeutic treatments aimed to prevent septic complications, reduce fistula discharge and ultimately heal fistulas.

Aim. The purpose of the study was to evaluate the efficacy and safety of biosimilar IFX (CT-P13) and adalimumab in active fistulizing perianal Crohn's disease (CD) in patients from the Subcarpathian Region (South-Eastern Poland).

Material and methods. Thirty patients with CD with perianal fistulas naïve to anti-TNF therapy were enrolled (13 females/ 17males) ranging from 18 to 64 years of age. Twenty-one were treated with biosimilar infliximab (CT-P13), nine were treated with adalimumab (ADA). The treated patients had ileal CD (4), ileo-colonic CD (13) or colonic CD (13). All of them received standard immunosuppression with no additional steroid therapy. Response was evaluated at week 16 and 40 after the first CT-P13 dose, and 16 and 40 weeks after the first ADA dose. Remission was defined as the complete closure of all fistulas and partial response as a reduction (≥50%) in the number of draining fistulas.

Results. Treatment outcomes with CT-P13 and ADA were both effective and similar in the percentage of patients with perianal fistula improvement, perianal fistula remission, no effect or observed adverse events.

Conclusion. In patients with active fistulizing CD, both CT-P13 and ADA were effective and safe, however a slight superiority of CT-P13 was visible.

Keywords. adalimumab, Crohn's disease, IFX, perianal fistula

Corresponding author: Anna Pękala, e-mail: apekala@onet.pl

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

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Introduction

The aetiology of perianal fistulas in Crohn's disease (CD) is still unclear. The presence of colonic and rectal disease represents the greatest risk factor for the development of perianal fistulas.[1] CD-associated fistulae appear as a fissure penetrating in the gut wall surrounded by granulation tissue with acute (neutrophils) and chronic (lymphocytes) inflammation. Their lumen is filled up by nuclear debris, sometimes erythrocytes.[2] The treatment of perianal CD requires a combined surgical and medical approach and should attempt to resolve and prevent septic complications, reduce fistula discharge with concurrent improvement of a patient's quality of life, and finally, the healing of fistulas. Treatment options depend on the severity of symptoms, fistula location, the number and complexity of fistula tracts, and the presence of rectal complications.

Aim

The purpose of the study was to evaluate the efficacy and safety of biosimilar IFX (CT-P13) and adalimumab (ADA) in active fistulizing perianal Crohn's disease (CD) in patients from Subcarpathian Region (South-Eastern Poland).

Material and methods

The human studies were approved by the Bioethical Commission of the University of Rzeszów (Resolution number 9/10/2016).

Thirty patients with CD with perianal fistulas naïve to anti-TNF therapy were enrolled (13 females/17males) ranging from 18 to 64 years of age. Twenty-one were treated with CT-P13 and nine were treated with ADA for the duration of twelve months. The treated patients had ileal CD (4), ileo-colonic CD (13) or colonic CD (13). All of them received standard immunosuppression with no additional steroid nor antibiotic therapy during biological treatment. Before including anti-TNF therapy, they received metronidazole with cyprofloxacin or other antibiotics according to the result culture of the content from the fistula for 4-6 weeks. The majority of patients in whom the inflammatory changes included the large intestine were additionally treated with mesalazine.[3]

All patients with fistulas underwent a pelvic MRI examination, on the basis of which the abscess was excluded and the course of the fistula was depicted. All those whose anatomical conditions allowed it to have a thread or seton attached to the fistula canal (25 patients). Assignment to the treatment was not performed in random fashion, but the decision was made based on overall disease activity. Therefore, patients with higher CDAI values were assigned to treatment with CT-P13. CT-P13 was used at a dose of 5 mg per kg / body weight scheduled at 0.2 and 4 weeks in induction therapy and every 8 weeks in maintenance therapy. ADA was given as a first dose of 160 mg, 80 mg after 2 weeks and then at a dose of 40 mg every 2 weeks. Response to the treatment was evaluated at week 16 and 40 after the first CT-P13 dose, and 16 and 40 weeks after the first ADA dose. Remission was defined as the complete closure of all fistulas and partial response as a reduction (≥50%) in the number of draining fistulas. Perianal disease improvement referred to reduction fistula drainage, reduction of bleeding, pain and excretion, edema, tenderness, and surrounding redness.

Simple descriptive statistical calculations were performed with the use of Statistica version 6.0. A statistical significance threshold of $P = 0.05$ was adopted.

Results

The basic characteristics of the study population are presented in Table 1. The efficacy of perianal fistula closure and improvement after the twelve month therapy with CT-P13 and ADA is presented in Table 2.

Table 1. Basic characteristics of the study population

Age	18-64 years
Sex	(13 females/17 males)
The form of the disease / number of patients:	ileal CD /4, ileo-colonic CD /13 colonic CD/ 13
Duration of the disease	1-7 years
Type of anti-TNF /group size	infliximab/21 adalimumab/ 9

Table 2. Perianal fistula closure and improvement after twelve month therapy with biosimilar infliximab (CT-P13) and adalimumab (ADA)

*Outcome	CT-P13	ADA
Perianal fistula improvement	6 patients (28.6%)	4 patients (44.4%)
Perianal fistula remission	10 patients (47.6%)	3 patients (33.3%)
No effect observed	5 patients (23.8%)	2 patients (22.3%)
Adverse events (perianal abscess)	9%- (2 persons)	11%- (1 person)

* No statistical significance was observed ($p > 0.05$)

Discussion

The perianal fistulas are an inconvenient complication of Crohn's disease (CD), significantly worsening the quality of life of patients. The risk of developing fistulas depends on disease location, being most frequent in colonic disease with rectal involvement. The cumulative incidence of perianal fistulising CD (pCD) is 12% after 1 year, and this doubles 20 years after diagnosis.[1,2] Disease lesions in the anus area in 27% of cases may be the first manifestation of the disease.[4]

Fistulas are a symptom of hollowing disease and risk factors for a more severe course of disease are: age under 40 years at the time of diagnosis, stenotic disease, involve-

ment of the upper gastrointestinal tract, need for corticosteroids on the first flare-up, lack of mucosal healing after induction of clinical remission, and smoking.[5]

Treatment of this form of the disease should be intensive from the very beginning to prevent deepening of tissue damage and abscess formation.

According to the guidelines set out in the 2014 European Society of Coloproctology Consensus, biological therapy with anti-TNFs is the gold standard for the treatment of fistulas in patients with CD.[6]

Our study assessed the efficacy of CT-P13 - biosimilar infliximab, and ADA treatment in 30 patients with active perineal disease in whom other pharmacological treatment options were exhausted. The use of IFX in this form of the disease is well established and this medicine is also used more frequently in cases of perinanal fistulas in our center, but significantly less clinical trials concern the use of ADA.

The efficacy of IFX in the treatment of perianal fistulas has been profoundly studied. In the first placebo controlled trial, an induction regimen induced closure of at least 50% of fistulas for at least 4 weeks in 56–68% of patients compared with 26% treated with placebo. Closure of all fistulas was achieved in 38–55% of patients on IFX.[6] The ACCENT II trial further evaluated IFX maintenance therapy for this indication. Week 14 responders to the induction regimen were randomized to further treatment with placebo or IFX 5 mg/kg every 8 weeks and 39% of patients who received IFX maintenance therapy had complete closure of all draining fistulas at week 54.[8] In the CHARM trial—a 56-week phase III trial to assess the efficacy of maintenance treatment with ADA among responders to induction treatment, a subgroup analysis in patients with draining fistula(s) at baseline showed complete fistula healing in 33% of adalimumab treated patients versus in 13% of placebo treated patients.[9] An open label extension of this trial showed sustained healing in 90% of patients on ADA treatment at 2 years follow-up. In further open label studies, adalimumab was effective in 23–29% of patients with fistulising CD who had lost response or become intolerant to IFX.[10,11]

It is emphasized that the approach to treatment of this form of the disease should be comprehensive.

A very important element of treatment is the determination of the anatomical course and type of fistula and exclusion of abscess. In our study, all patients had a pelvic MRI scan, which is considered the preferred method that accurately visualizes the anal sphincter and the pelvic floor muscles as well as the fistula tracts and abscesses. In addition, the MRI scan can identify clinically 'silent' abscesses and luminal inflammation.[12-14]

Patients with abscesses were first treated surgically with drainage, only after the abscess was resolved they were qualified for biological therapy.

The surgical procedure also involved insertion of a thread or seton into the fistula canal in order to prevent abscess formation. This procedure was used in the majority of patients in whom the anatomical conditions allowed it.

It is believed that non-cutting seton placement is very useful in order to prevent (recurrent) abscess formation.[14] In contrast, a disadvantage of setons is that the fistula tract cannot 'close' with the seton in place. The optimal timing for seton removal is not well established.[14] In accordance with the principles of a comprehensive approach to treatment, the studied group received all the preferred methods of therapy, including immunosuppressive treatment.

Considering the studies carried out so far, anti-TNF and thiopurine combination therapy may lead to higher fistula healing response and closure rate compared to monotherapy.[5,16] However, the results of all tests carried out are not compatible, e.g. a subgroup analysis of the ACCENT II trial found that concomitant immunosuppressants did not improve response rates to IFX at 1 year.[17] While another recent studies suggest a clear association between combination therapy and fistula closure, nevertheless, the gain with combination therapy is of particular in patients with proctitis.[18,19]

An additional argument for combining combination therapy is to reduce the production of anti-infliximab antibodies, which reduces the percentage of secondary loss of response to treatment; in the case of ADA, clinical trials did not show such an advantage.[20]

According to the guidelines, all patients also received antibiotic therapy (ciprofloxacin and metronidazole) consistent with the result of culturing the content obtained from the fistula, which was carried out from 4 to 6 weeks depending on the tolerance. All patients achieved a reduction in fistula drainage, but not fistula healing; in patients in whom the time between the end of antibiotic therapy and the initiation of biological treatment was prolonged, an increase in secretion was observed.

These observations are consistent with clinical trials that evaluated the efficacy of longer treatments for metronidazole and ciprofloxacin (6 to 8 weeks) and a high frequent relapse upon discontinuation and side effects occurring was reported.[20-22] In turn, studies evaluating ciprofloxacin-combined therapy and anti-TNF drugs (IFX and ADA) showed reducing fistula drainage but not fistula healing.[23,24]

We have demonstrated that the combination of thiopurins and CT-P13 or ADA therapy preceded by antibiotic therapy and surgical treatment (abscess drainage, seton fistulae) gives slightly higher efficacy in fistula healing when IFX was used 47.6% vs. 33%. The lack of any response was noted in a similar percentage of cases, 23.8% and 22.3%, for CT-P133 and ADA, respectively.

Despite the limitation of our study, which is a relatively small number of patients and the prevalence of infliximab-treated patients, the results obtained coincide with other studies. On the basis of the analysis, the risk factor of non-response was not identified, whereas it was observed that complex and multiple perirectal fistulas were a risk factor for lack of healing but no lack of response (patients achieved a reduction in secretion by at least 50%).

Conclusion

In patients with active fistulizing CD, both biosimilar IFX and adalimumab were effective and safe, however, a slightly better outcome with biosimilar IFX was observed. Treatment outcomes with biosimilar IFX and adalimumab were both effective and similar in the percentage of patients with perianal fistula improvement, perianal fistula remission, no effect or observed adverse events. The results obtained in this study concur with other published trials.

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ORIGINAL PAPER

Aleksandra Barańska [1(BCDEG)], Aleksandra Maziarz [2(DEFG)], Jacek Roliński [1(AH)], Janusz Klatka [3(BH)], Katarzyna Dudzińska-Ćwiek [3(B)], Jacek Tabarkiewicz [2,4(ADEFG)]

Expression of heat shock protein 70 in the tissue of patients with laryngeal squamous cell carcinoma

[1] Chair and Department of Clinical Immunology, Medical University of Lublin, Lublin, Poland
[2] Institute of Experimental and Clinical Medicine, Department of Immunology, Faculty of Medicine, University of Rzeszow, Rzeszow, Poland
[3] Chair and Department of Otolaryngology and Laryngological Oncology, II Faculty of Medicine with English Language Division, Medical University of Lublin, Lublin, Poland
[4] Centre for Innovative Research in Medical and Natural Sciences, Faculty of Medicine, University of Rzeszow, Rzeszow, Poland

ABSTRACT

Introduction. Laryngeal squamous cell carcinoma (LSCC) is a common type of head and neck malignancy. Because of unsatisfactory results of therapy, development of new strategies for LSCC treatment is needed. It is believed that heat shock protein 70 (HSP70) is involved in pathogenesis of LSCC. Thus, targeting HSP70 seems to be promising strategy for laryngeal cancer treatment.

Aim. The aim of the study was to assess the HSP70 concentration in laryngeal squamous cell carcinoma specimens and its correlation with tumor volume and TNM staging.

Material and methods. An ELISA method and a Bradford protein assay were used to evaluate the HSP70 concentration in peripheral blood cells, tumor tissue and lymph nodes from the patients suffering from LSCC.

Results. We demonstrated that the HSP70 concentration is significantly different between examined compartments. The highest level was observed in peripheral blood, while the lowest was in the lymph nodes. The HSP70 expression was correlated to tumor volume.

Conclusion. Our results showed varied expression of HSP70 in tissue from patients with LSCC, but there was no association between HSP70 concentration and TNM staging. Currently, application of HSP70 inhibition as a LSCC treatment could be rather associated with systemic blocking of this molecule than target inhibition in tumor tissue. However, further analysis on a larger group of patients is needed.

Keywords. HSP70, LSCC, treatment

Corresponding author: Aleksandra Maziarz, Warzywna, e-mail: a.maziarz01@gmail.com

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

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The list of abbreviations:

CD – cluster of differentiation, **DCs** – dendritic cells, **HSPs** – heat shock proteins, **HSP27** – heat shock protein 27, **HSP70** – heat shock protein 70, **HSP72** – heat shock protein 72, **HSP90α** – heat shock protein 90α, **HSP90β** – heat shock protein 90β, **LSCC** – laryngeal squamous cell carcinoma, **PBMC** – peripheral blood mononuclear cells, **SCC** – squamous cell carcinoma

Introduction

Laryngeal squamous cell carcinoma (LSCC) is one of the most common types of head and neck malignant tumors. The incidence of LSCC remains high, with high rates of metastasis and recurrence. Laryngeal cancer occurrence is related to age, most frequently affecting individuals in sixth or seventh decades of life. It affects male more often than females.[1–3] There are several risk factors for laryngeal cancer; two of the major risk factors are tobacco smoking and alcohol consumption. Other risk factors for LSCC include occupational agents, such as asbestos, polycyclic aromatic hydrocarbons, solvents and dust. The role of other factors, like viral infections, genetics and environmental influence remains unclear.[1]

LSCC treatment includes several methods, such as laryngectomy, radiotherapy and chemotherapy or combination of these. Treatment modality is determined by tumor location and staging. However, despite of application of different treatment methods, the 5-year survival rate in advanced LSCC is still unsatisfactory.[1] Therefore, a search for new possibilities for laryngeal cancer therapy is needed.

Resistance of tumor cells to therapy may be caused by overexpression of heat shock proteins (HSPs).[4] Many previous studies are focused on the role of HSPs in carcinogenesis. Due to the fact that HSPs are able to inhibit apoptosis of cancer cells, the inhibition of HSPs activity is considered as a cancer treatment.[5]

It was shown that different types of HSPs were overexpressed in different types of tumors. Moreover, increased expression of these proteins was associated with histological grade, recurrence and metastasis of malignant tumors.[6] Overexpression of heat shock protein 70 (HSP70) was observed in LSCC among head and neck malignant tumors.[7] However, the role of HSP70 in LSCC is not fully understood.

HSP70 as a molecular chaperone is involved in process of folding of newly synthesized proteins, assembly of protein complexes and transmembrane transport of proteins. HSP70 enhanced the cell tolerance to effects of stress condition, such as increased concentration of unfolded and denatured proteins.[5] It has long been known that HSP70 is able to inhibit apoptosis and increase an oncogenic potential of tumor cells.[5,8]

Aim

The aim of the study was to determine the concentration of HSP70 in blood cells, tumor tissue and lymph nodes of patients suffered from laryngeal squamous cell carcinoma and to determine the correlation between expression of HSP70 and type of tissue, tumor volume and TNM staging.

Material and methods

Peripheral vein blood samples, fragments of tumor and lymph nodes were obtained from 16 patients suffering from laryngeal squamous cell carcinoma who underwent tumor resection in Department of Otolaryngology and Laryngological Oncology, Medical University in Lublin. The material was collected from patients with tumor in Grade 2 or Grade 1 (only one case). The patient characteristics are summarized in Table 1.

Table 1. Characteristics of the patient group

Patient	Tumor grade	TNM staging
1	G-2	T2N3M0
2	G-2	T3N1M0
3	G-2	T3N2M0
4	G-2	T3N0M0
5	G-2	T3N0M0
6	G-2	T3N3M0
7	G-2	T3N0M0
8	G-1	T3N2M0
9	G-2	T3N1M0
10	G-2	T3N0M0
11	G-2	T3N0M0
12	G-2	T4N0M0
13	G-2	T4N2M0
14	G-2	T3N1M0
15	G-2	T4N2M0
16	G-2	T3N2M0

Peripheral blood mononuclear cells (PBMC) were isolated by gradient centrifugation using Gradisol L (Aqua Medica, Poland). Cells were washed twice with phosphate buffer saline (PBS) w/o Mg^{2+} and Ca^{2+} (PAA Laboratories GmbH, Austria). Lymph node and tumor tissue fragments were homogenized using tissue knives (Medicon, Dako, Denmark) and MediMachine whipper (Dako, Denmark). Cells were suspended in cryomedium containing 70% of RPMI 1640 (PAA, Austria), 20% of human albumin (Baxter, Austria) and 10% of dimethyl sulfoxide (DMSO) (ICN Polfa Rzeszów, Poland) and cryopreserved in liquid nitrogen vapor. Cells were thawed using CTL-Test Medium (C.T.L Ltd, USA) and supplemented with 10% of CTL-Wash Supplement (C.T.L Ltd, USA) and after subsequent PBS wash they were suspended in 1 mL of PBS. Cells lysates were prepared by freezing cells in -80°C

Table 2. The standardized HSP70 concentration in examined tissue

	Mean ± standard deviation [pg/ml]	Median [pg/ml]	Minimum [pg/ml]	Maximum [pg/ml]
HSP70 PBMC	2535.37±816.23	2658.65	572.27	4017.23
HSP70 lymph node's cells	1435.34±1246.28	1218.56	31.60	4107.66
HSP70 tumor's cells	1822.09±1394.04	2088.43	121.64	3603.29

Table 3. Correlation between concentration of HSP70 in different tissue and tumor volume (*p < 0.05)

Pair of variables	Spearman's R	p
HSP70 PBMC [pg/ml] & tumor volume [cm^3]	-0.195	0.523
HSP70 lymph node's cells [pg/ml] & tumor volume [cm^3]	0.826	0.011*
HSP70 tumor's cells [pg/ml] & tumor volume [cm^3]	0.226	0.559

and thawing in water bath at 37°C five times. Samples were centrifuged and the supernatant was collected.

Total protein concentration was determined by Bradford protein assay. The absorbance was measured using Spectrophotometer SmartSpecTM 3000 UV/VIS (Bio-Rad) at a wavelength of 595 nm. HSP70 expression was examined using commercial kit for Sandwich ELISA Human/Mouse/Rat Total HSP70 Immunoassay (R&D Systems, USA). The procedure was performed according to the protocol. The plate was analyzed using a Multilabel Plate Reader Victor 3^{TM} (Perkin-Elmer) at a wavelength of 450 nm. HSP70 concentration was standardized in reference to 1 mg of total protein.

Statistical analysis of the data was performed using Statistica 9.0 PL. Wilcoxon signed-rank test, Spearman's rank correlation coefficient, median's test for multiple group comparison and Friedman ANOVA test were used for the analysis. Data are presented as mean ± standard deviation, median, minimum and maximum result. Results were considered as statistically significant at the significance level $p < 0.05$.

Results

The expression of HSP70 was determined using an ELISA assay and then HSP70 concentration was standardized in reference to 1 mg of total protein. The highest concentration of HSP70 was observed in PBMC, while the lowest concentration was observed in the lymph node cells (Table 2 and Figure 1). With the use of Friedman's ANOVA test, we did not find any statistically significant differences between examined tissue (χ^2 ANOVA=2.8; p=0.25). The post-hoc Wilcoxon signed-rank test showed statistically significant differences between HSP70 concentrations in PBMC and lymph node cells (p=0.050). There were no significant differences between HSP concentrations in other tissue (Figure 1).

Statistical analysis of correlation between concentration of HSP70 in different tissues and tumor volume was performed using Spearman's rank correlation test. We observed statistically significant positive correlation between HSP70 concentration in lymph node cells and tumor volume (R=0.826, p=0.011). Correlations between HSP70 concentrations in white blood cells and tumor cells were nonsignificant (Table 3).

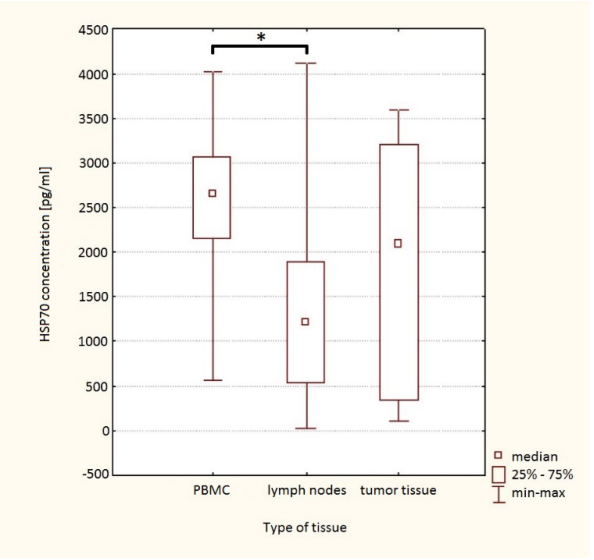

Fig. 1. HSP70 concentration [pg/ml] in PBMC, tumor tissue and lymph node's cells (*p < 0.05, Wilcoxon signed-rank test)

Comparison between HSP70 concentration and TNM staging performed using the median's test for multiple group comparison did not show any significant associations. However, because the study group was relatively small, it can be assumed that a study performed on a larger group of patients may reveal a different result.

Discussion

Heat shock proteins play a protective role in organisms and support adaptation to extreme conditions. HSPs represent the evolutionary old defensive system in all living organisms. However, higher expression of HSPs occurs also in cancer tissue. Due to HSPs overexpression and their possible role in cancer pathogenesis, inhibition of these proteins has been considered as a promising strategy in cancer treatment.[9]

HSP70 has the most conservative structure among the heat shock protein family. It plays a pivotal role in protein folding process and maintenance of genome stability under stress conditions. Moreover, HSP70 indicates cytoprotective features and it has capability to inhibit apoptosis. As a highly protective protein, HSP70 may lead to survive of abnormal and defective cells.[10,11] Enhanced expression of HSP70 is observed in cancer cells as well. It is believed that its overexpression is related to carcinogenesis and cancer progression.[11,12] Furthermore, high expression of HSP70 in cancer tissue is also associated with metastasis and therapeutic resistance, and consequently shorter patient survival prognosis.[11,13,14]

Several studies have shown that increased level of HSP70 expression was associated with a poor clinical outcome and poor response to therapy in colorectal cancer, breast cancer and head and neck squamous cell carcinoma (SCC).[14–18] It has been suggested that inhibition or knockdown of HSP70 applied as a cancer treatment may contribute to tumor regression.[11] Chen et al. demonstrated that viral proteins may suppress tumor growth via interaction with HSP70. Overexpressed viral protein formed a complex with HSP70 causing inhibition of its activity, which consequently led to inhibition of cancer cell viability and induction of cancer cell death.[12]

In contrast, it has been demonstrated by Enomoto et al. and Gong et al. that HSP70 is able to induce antitumor immune response and prevent the tumor growth. They used HSP70 isolated from tumor-dendritic cell fusion to vaccinate mice and showed that HSP70 stimulated dendritic cells (DCs) maturation and T cell proliferation. HSP70 induced T cell-mediated immune response. It significantly increased the proliferation of CD8+ lymphocytes and induced the effector and memory T cells.[19,20] Similarly, a study performed by Chen et al. showed that HSP70 from tumor cells is involved in initiation of antitumor immunity by activation of DCs and monocytes/macrophages, and it is involved in enhancement presentation of tumor antigens to T lymphocyte. Moreover, they observed that stress conditions, such as hyperthermia, accelerated release of HSP70 by tumor cells and enhanced stimulation of antitumor immune response.[21] On the basis of these studies, it is believed that HSP70 and other HSPs may be a promising tool in the development of anticancer vaccines.

The expression of HSP70 in laryngeal cancer tissue has been studied previously. Xu X et al. evaluated the expression level of heat shock proteins (HSP90α, HSP90β, HSP70 and HSP27) in laryngeal carcinoma and normal laryngeal mucosa. They perceived fivefold overexpression of HSP90α and HSP70 in carcinoma tissue, suggesting that HSP90α and HSP70 can be involved in the pathogenesis of laryngeal cancer.[22] Yang et al. also analyzed expression of HSP70 in specimens of human laryngeal squamous cell carcinoma and specimens of para-carcinoma. They observed increased expression of HSP70 in carcinoma tissue compared with para-carcinoma tissue, which was significantly correlated with the differentiation of LSCC.[23] Xu J et al. showed that expression of HSP70 is correlated with histological grade of LSCC. Using immunohistochemistry methods, they observed HSP70 presence in 96% of samples of tumor tissue. The level of HSP70 expression was significantly lower in early stages of LSCC than in late stages.[6]

According to mentioned studies, the concentration of HSP70 was increased in LSCC tissue compared with samples of healthy tissue. Therefore, we can suppose that HSP70, such as HSP90 can be involved in pathogenesis of laryngeal squamous cell carcinoma.

Results obtained in our study revealed that the level of expression of HSP70 in patients with LSCC is various in different types of tissue. The highest expression of HSP70 was observed in white blood cells, while the lowest was observed in lymph node's cells. Nevertheless, HSP70 expression did not show cancer tissue tropism, which would be desirable for potential application of HSP70 inhibition in cancer therapy.

Additionally, in present study we surprisingly observed a strong positive correlation between HSP70 concentration in lymph node's cells, which was the lowest among examined compartments, and tumor volume. Because HSP70 is involved in delivery of antigens to antigen-presenting cells and therefore stimulate an adaptive response,[24] the low concentration of HSP70 in lymph nodes draining tumor could be associated with decreased activation of tumor specific lymphocytes and subsequently increased tumor progression. On the other hand, we found a positive correlation between concentration of HSP70 in lymph nodes and tumor volume, what could be associated with its protective role and anti-apoptotic functions, HSP70 can increase oncogenic potential of cells and it can contribute to tumor progression.[5] The positive correlation between concentration of HSP70 in lymph nodes and tumor volume could be theoretically explained by the fact, that HSP70 is transferred from tumor to lymph nodes via lymph and represent higher concentration of protective HSP70 in malignant tissue.

Previous studies have demonstrated that HSP70 expression correlates with the clinical stage of oral SCC. Tavassol et al. observed that survival of patients with T2 tumors and positive expression of HSP70 was eightfold higher than in the case of patients with T2 tumors and the lack of expression of HSP70. However, the expression of HSP70 affects survival only in the early stages of the disease. The level of expression of HSP70 had no prognostic significance for T3 and T4 tumors.[25] Furthermore, Taghavi et al. noticed a significant correlation

between expression of HSP70 and clinical stage, lymph node metastasis and tumor volume, whereas no association with histological grade was found.[26] In contrast, a study performed on esophageal SCC showed greater expression of HSP70 and HSP27 in tumor tissue, which was related to follow-up of patients. HSP70 and HSP27 expression exhibited a significantly better prognosis. While there was no correlation with other clinical parameters such as gender, age, lymph node status and tumor differentiation.[27] In the present study, the analysis of the correlation between HSP70 concentration and TNM staging did not show any statistically significant associations. However, it is worth noting that the limiting factor of the analysis was a small study group. For this reason we can suppose that a study conducted on a larger group of patients may reveal a different result.

Conclusions

to summarize, in the present study we observed a strong positive correlation between HSP70 concentration in lymph node cells and tumor volume. The level of expression of HSP70 in different tissue from LSCC patients was varied, and did not show cancer tissue tropism. The HSP70 expression did not correlate with TNM staging, however, due to the fact that in our study group was only one case with T2 tumor, it can be assumed that the result of study performed on a larger group of patient would be different. Nevertheless, on the basis of current results, the application of HSP70 inhibition as a LSCC treatment could be rather associated with systemic blocking of this molecule than target inhibition in tumor tissue. Moreover, previous studies have shown that the expression of HSP70 is higher in patients with LSCC compared to healthy ones. Because of that, it seems necessary to compare results obtained in our study with the control group. Therefore, future studies should focus on the influence of other HSPs on LSCC.

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ORIGINAL PAPER

Zuzanna Bober [1 (ABG)], Piotr Bar [2 (ABG)], Grzegorz Pasternak [3 (ABG)], Sabina Galiniak [4 (ABDFG)], Dorota Bartusik-Aebisher [4 (ABDFG)], Rafał Filip [3 (ABDFG)], David Aebisher [5 (ABDFG)]

Infliximab MRI relaxation time in solution

[1] Department of Electroradiology, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[2] Clinical Department of Pathomorphology, Clinical Hospital No. 2, Rzeszów, Poland
[3] Department of Gastroenterology, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[4] Department of Biochemistry and General Chemistry, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[5] Department of Photomedicine and Physical Chemistry, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland

ABSTRACT

Introduction. The use of Quantitative Magnetic Resonance Imaging to drug monitoring in vitro or in vivo can provides a powerful means to map the effects of drugs on tissue activity.

Aim. The purpose of this study was to measure relaxation time of antibody phantom. For this purpose, infliximab sample was used.

Material and methods. The selected methods to detect relaxation time of antibody was Magnetic Resonance Imaging technique. We detected spin-lattice (T_1) relaxation time and discuss differences where compare to water.

Results. The measurements of spin-lattice (T_1) relaxation time showed significant differences. The results obtained in phantom indicate that we can use this result for measurements of relaxation time *in vitro*.

Conclusion. Infliximab is approved for severe cases of rheumatoid arthritis, together with methotrexate, for pronounced psoriasis and psoriasis-arthritis, ankylosing spondylitis as well as for chronic inflammatory bowel disease. We conclude, that Quantitative Magnetic Resonance Imaging can be used to monitor drug effects.

Keywords. infliximab, magnetic resonance imaging, relaxation time

Introduction

Quantitative Magnetic Resonance Imaging (QMRI) has already brought advances in diagnostic research.[1-5] Proton ^{1}H MRI has been already used to monitor a number of pharmaceutical processes e.g. to monitor the dosage form *in vivo* and correlate with *in vitro* behavior. The use of QMRI to drug monitoring *in vitro* or *in vivo* can provides a powerful means to map the effects of drugs on tissue activity. Additionally, QMRI is important applications in pharmacological research. While standard MRI can provide basic information regarding tumour, the quantified QMRI can evaluate the effectiveness of drug therapy. Of particular interest are changes in drug relaxivity which are correlated

Corresponding author: David Aebisher, e-mail: daebisher@ur.edu.pl

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

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with drug uptake.[5-10] The aim of our study was to determine whether changes in spin-lattice relaxation time T_1 and spin-spin relaxation time T_2 allow monitoring the treatment with infliximab antibody. T_1 and T_2 in MRI are functions of spin density and also instrumental parameters such as the pulse sequence timing and slice selective sensitivity profile. At the same time valuable physiological information can be extracted from infliximab, while quantitative investigations of dynamics of drug delivery and drug effects is crucial for the development of effective therapy. Therefore, there is a growing interest in using MRI to examination of antibody drug such as infliximab.[10-15]

Material and methods

We have studied Infliximab using Magnetic Resonance Imaging techniques. Measurements of spin-lattice T_1 relaxation time were made using a 1.5 Tesla Magnetic Resonance Imager (Optima MR360 Advance, General Electric Healthcare). Three prepared phantoms:

(1) infliximab solution
(2) water
(3) glue

were placed in the magnet. The samples were then scanned using Fast Spin Echo sequences with a coronal projection using a 4-channel small flex coil with a matrix size of 320 × 224, a field of view of 10 cm × 10 cm, and a slice thickness of 2 mm. The T_1 relaxation time was measured using the saturation recovery method with a Time to Echo (TE) TE=3 ms and Time to Repetition (TR) TR= 50 ms, 100 ms, 200 ms, 300 ms, 500 ms, 700 ms, 1000 ms, 1500 ms, 2000 ms, 2500 ms, 3000 ms, 5000 ms, 10 000 ms and 15000 ms. Based on the generated image sequence, the MRI signal was collected from the region of interest that covered the same area in each sample.

Results

Here, we have investigated the QMRI response to infliximab. Infliximab is widely applied as a pharmacological drug. Pharmacological QMRI can tracks signal changes that reflect drug challenges and may be considered as a surrogate for changes in the gastroenterological processes. The QMRI images of 1) infliximab solution, (2) water and (3) glue showed the homogenous solutions of (1-3) phantoms. We observed that the relaxation time is increasing in direction (3) glue> (2) infliximab and (3) water. The changes in values due to proton density in phantoms caused changes in relaxivity values. The region of interest (ROI) in phantoms was selected in the same area in samples. The images became darker due to shorter T_1 values, that were associate with the loss of protons, decrease in water concentrations within the ROI. The measured values of relaxation time of (1), (2) and (3) phantoms are presented in Table 1.

Table 1. Relaxation time

T_1-Water	T_1-Infliximab	T_1-Glue
3390 ms	2545 ms	2790 ms

Discussion

Infliximab is monoclonal antibody which contains a human constant region and a mouse-derived murine variable region. Infliximab is specific for human tumor necrosis factoralpha (TNFα). There is a rapid increase in the applications of MRI for molecular and cellular imaging in vivo an ex vivo. However, to study the cellular details of drug treatment, high resolution MR and long acquisition time are needed. MRI shows to be a useful technique for evaluation of infliximab. Studies in recent years have shown that MRI is essential for assessment of drug response. Therefore, in recent years, the use of MRI in patients with Crohn's disease has increased.[1-46] MRI is sensitive to the anti-inflammatory effects of infliximab.[1] MRI of Crohn's disease patients identify predictors of deep remission on long-term maintenance anti-tumor necrosis factor α therapy.[2-18] Magnetic resonance diffusion-weighted imaging was used after infliximab induction therapy in patients with Crohn's disease.[19] Tumor necrosis factor antagonists can induce mucosal healing in patients with Crohn's disease.[20] Anti-tumor necrosis factor therapy heals many Crohn's disease.[21,22] To evaluate the role of pelvic MRI in diagnosis and assessment of combined surgical and infliximab treatment of Crohn's disease.[23] MRI is used to assess the outcome of infliximab therapy in patients with perianal fistulizing Crohn's disease.[24-34] Infliximab therapy, including loss of enhancing nodules and loss of meningeal enhancement.[35] MRI of infliximab, was used to evaluate the frequency and location of erosions.[36] MRI seems to be interesting for objective therapeutic evaluation and monitoring of patients with spondyloarthropathy.[37,38] MRI examination was helpful in documenting the effect of treatment over this short period.[39] Patients who received infliximab therapy showed a decrease in spinal inflammation as detected by MRI.[40] In refractory rheumatoid arthritis patients, the addition of infliximab therapy may result in clinical, laboratory and magnetic resonance imaging improvement.[41] MRI may have significant implications for the optimal use of expensive biologic therapies.[42-44] MRI and clinical evaluation were performed before and after infliximab infusions.[45,46]

Conclusion

Infliximab is approved for severe cases of rheumatoid arthritis, together with methotrexate, for pronounced psoriasis and psoriasis-arthritis, ankylosing spondylitis as well as for chronic inflammatory bowel disease. We conclude, that QMRI can be used to monitor drug effects.

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ORIGINAL PAPER

Jacek Łukasiewicz [1 (ABCDFG)], Marcin Stencel [1 (ABCDFG)], Adam Raczkowski [1 (ABCDFG)], Andrzej Cwynar [1 (DGH)], Wiktor Cwynar [1(DGH)], Tomasz Saran [2 (ABCDFG)], Anna Mazur [2 (BCDEFG)]

Personality correlates in the tendency to incur debt in patients with and without osteoarthritis

[1] University of Economics and Innovation, Lublin, Poland
[2] Department of General and Neuro Rehabilitation, Witold Chodźko Institute of Rural Health, Lublin, Poland

ABSTRACT

Introduction. Osteoarthritis is the most common disease of the musculoskeletal system. Osteoarthritis progresses slowly, but with time it results in movement disability and chronic pain. Its progression is also significantly associated with personality functioning and the ability to make rational life choices regarding various aspects of human life, including the economic sphere.

Aim. The aim of the research was to determine how personality correlates with a tendency towards indebtedness between patients with and without osteoarthritis.

Material and methods. The researched sample consisted of two groups - 50 patients diagnosed with osteoarthritis hospitalized in the Department of General and Neuro Rehabilitation in the Institute of Rural Health in Lublin and 50 healthy people. The KOS-B Questionnaire, the IVE Questionnaire, the SES Scale, the SPP-25 Scale, the Delta Questionnaire and the APSZ questionnaire were used in the research.

Results. In the group of patients with osteoarthritis, the leading correlates of indebtedness are: assessment of the stress situation related to the current economic situation seen as a threat, and perceiving it as harm or loss. In the healthy group, the leading correlates of incurring financial liabilities are impulsiveness, self-esteem, assessment of the stress situation related to the current economic situation seen as a threat, openness to new experiences and sense of humor, empathy, and the need for social approval.

Conclusion. In the group of patients with osteoarthritis, the factor conducive to incurring financial liabilities is the assessment of the stressful situation related to the current economic situation seen as a threat, while perceiving it as harm or loss leads to abandoning reliance on credit. In the healthy group, the factors that favor this type of activity are impulsiveness, self-esteem, the assessment of the stress situation related to the current economic situation seen as a threat and the need for social approval; the factors protecting against indebtedness in healthy persons are openness to new experiences and sense of humor as well as empathy.

Keywords. life on credit, osteoarthritis, personality

Corresponding author: Jacek Łukasiewicz, e-mail: jacek.lukasiewicz@wsei.lublin.pl

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

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Introduction

Osteoarthritis is the most common disease of the musculoskeletal system caused by the disorder of surface smoothness and loss of articular cartilage. Structurally, the disease is characterized by the formation of subchondral cavities, bone excrescences and excessive calcification of the subchondral bone. Primary degenerative disease results from involutional processes, however, overloading of motor organs and metabolic disorders accelerate natural degenerative processes within the joint.[1] As a result of pain and movement disorders, changes in the tissues surrounding the affected joint become evident over time, which leads to muscular atrophy, deformities and contractures of the entire limb, similarly as in the case of inflammatory diseases of the joints. Osteoarthritis progresses slowly, but over time it causes physical disability and chronic pain and socio-economic exclusion, which is also significantly associated with personality functioning and the ability to make rational life choices regarding various aspects of human life, including the economic sphere.[2]

The issue of personality is one of the most interesting and at the same time the most controversial issues in psychology. Different psychological approaches capture the personality of a person in a very diverse way. All authors try to explain the mechanisms that affect the functioning of people, and at the same time determine the uniqueness of the individual and the sense of its identity. It should be noted, however, that no specific personality definition can be used in a universal way. This means that the definition of personality depends strictly on the adopted theoretical assumptions. Therefore, it should be assumed that the human personality comprises a set of descriptive features that characterize a person in terms of dimensions occupying a central position within the applied theory.[3]

This work is based on a cognitive approach to a personality, which is characterized by eclecticism and deals with a human-specific way of constructing events, the personal level of integration of one's behavior and the style of action. In terms of this concept, what gives meaning to human experience is the concept of "Self", whereas personality is treated as a system of personal knowledge, used in the interpretation of experience and control of behavior.[4,5] The cognitive approach to personality strongly concentrates on the image of one's "self", because this concept is responsible for giving a comprehensive sense to our experiences and for determining our attitude to the world through subjective control and self-regulation. Thanks to these processes, a human being can exert the desired influence on the course of events and ensure their own behavior an adequate level of internal integration.[6]

Thanks to the theoretical framework determined by cognitive concepts, it was possible to include personality as a variable in the study of relationships of individual personality traits with various aspects related to personal finance management, with a particular focus on financial liabilities. Works devoted to this subject indicate the existence of two major dimensions representing different aspects of attitudes towards money. The first is the belief in the symbolic nature of money and refers to their psychological functions, while the second dimension is related to the management of money in accordance with their economic functions.[7]

As Gąsiorowska points out, belief in the symbolic meaning of money is related to materialism, neuroticism, anxiety (understood as a trait and state), low agreeableness and extraversion, external locus of control and low self-esteem, as well as assessing one's financial situation as bad. In turn, the attitude focused on money management coincides with completely different variables. The only personality trait linked to this attitude is conscientiousness, while the remaining correlates are typically economic behaviors (e.g. the number of bank accounts, payment cards or the value of savings).[8]

Interesting results on personality correlates of financial management are also provided by Watson, Jones and Morris.[9] They show that the desire to have money is an integral part of the narcissistic development of the "Self". People with a high level of narcissistic tendencies covet money, consider it a sign of prestige and success in life, but also experience fear that they will lack resources for a decent life. The results of Lester, Yang and Spinelli research show additionally that people with high levels of anxiety (understood as a trait) may have problems with organizing their budget, they also perceive money as a source of strength and prestige in the eyes of other people. They are also more uncertain and suspicious and in financial decision-making situations compared to people with low levels of anxiety as a trait. The above considerations indicate a significant relationship between specific personality traits and selected aspects of personal finance management. Awareness of these links can help you understand how to budget and control your personal financial resources. It should also be the basis for broadly understood financial education.[10]

Aim

The aim of the study was to determine the personality correlates of life on credit of patients with osteoarthritis and healthy people.

Material and methods

The research sample was collected on the basis of intentional choice. It consists of two groups - 50 patients with a diagnosis of osteoarthritis hospitalized in the Department of General and Neuro Rehabilitation in the Institute of Rural Health in Lublin and 50 healthy people. Detailed characteristics of the subjects are presented in Table 1.

Table 1. Sociodemographic characteristic of the researched groups

Variables		Group			
		patients		health people	
		N	%	*N*	%
Sex	woman	25	50.0	25	50.0
	man	25	50.0	25	50.0
Marital status	single	6	12.0	10	20.0
	married	28	56.0	39	78.0
	divorced	5	10.0	1	2.0
	widowed	11	22.0	0	0.0
Education	elementary	10	20.4	1	2.0
	vocational	11	22.4	2	4.0
	secondary	17	34.7	31	62.0
	higher	11	22.4	16	32.0
Place of residence	village	19	38.8	21	42.0
	city up to 100 thousand residents	16	32.7	15	30.0
	city over 100 thousand residents	14	28.6	14	28.0

Source: Our own research results.

The average age of the patients undergoing rehabilitation was over 61 years (M = 61.27, SD = 16.00), and healthy persons approximately 60 years (M = 59.95; SD = 9.93). Both the clinical group and the healthy population were homogenous in gender. Each group is dominated by married people, who have completed secondary education and come from rural areas.

The research was carried out using the Stress Appraisal Questionnaire, version to assess dispositional stress appraisal (KOS-B) by D. Włodarczyk and K. Wrześniewski, IVE Impulsiveness Inventory by HJ Eysenck and SBG Eysenck in the Polish adaptation by A. Jaworowska, SES M. Rosenberg Self-Esteem Scale in the Polish adaptation by I. Dzwonkowska, K. Lachowicz-Tabaczek and M. Laguna, the Resiliency Assessment Scale (SPP-25) by Ogińska-Bulik and Juczyński, the Delta Questionnaire by R.Ł. Drwal and the Psychosocial Aspects of Debt Questionnaire (APSZ).[11-15]

The KOS-B questionnaire is used to measure a dispositional appraisal of a stressful situation. It comprises 35 adjectives that make up 4 scales: 1) threat, 2) harm/loss, 3) challenge-activity, 4) challenge-passivity. Subjects give answers based on a 3-point scale. The tool is designed for individual and group research of adolescents and adults, both healthy and sick.[11]

The IVE questionnaire was constructed to assess three personality traits: impulsiveness, venturesomeness, and empathy. It consists of 54 questions to be answered with Yes or No. It can be administered in research as well as in the individual diagnosis.[12]

The SES scale is the most commonly used tool for self-esteem measurement. SES can be used in studies of adolescents and adults. SES consists of 10 statements. Subject answers on a four-point scale depending on the degree to which he or she agrees with the statements.[13]

The SPP-25 scale was based on the concept of resilience, which recognizes this resource as a personality feature, which is a self-regulatory mechanism, including cognitive elements typical of beliefs and expectations, emotional characteristic of emotional stability and readiness to undertake diverse and effective strategies to deal with the encountered difficulties and the tendency to seek new experience. The tool includes 5 scales: 1) persistence and determination in action, 2) openness to experience and sense of humor, 3) personal competences to cope and tolerance of negative emotions, 4) tolerance of failure and perceiving life as a challenge, 5) optimistic attitude to life and the ability to mobilize oneself in difficult situations. Participants respond to 25 items using a five-point Likert scale. It is intended for adults, both healthy and sick.[14]

Delta questionnaire is used to measure the locus of control - astable and generalized belief of an individual regarding their own location of reinforcement control. People with internal locus of control are convinced that they control what is going on around them, while people with an external locus of control believe that they have no influence on the course of actions related to them. The tool, apart from the main scale, additionally contains a scale for measuring the level of social approval.[15]

The APSZ survey was constructed for the needs of the presented research and concerns various social situations related to incurring financial liabilities. It consists of 17 questions concerning subjects' current financial situation, current or past financial obligations, a self-description of the so-called economy, conviction about having basic knowledge about financial management and reliability in repayment of one's financial obligations. When answering the eight questions, the subjects choose the correct answer, and in nine items they give answers on the 5-point Likert scale.

The research was carried out in 2018 after having obtained the consent of the Bioethical Commission of the Witold Chodźko Institute of Rural Medicine in Lublin. The research was voluntary, individual and anonymous. It was conducted in accordance with the Declaration of Helsinki from 2013.

The subjects were informed about the purpose and course of the research, the confidentiality of the collected data and they consented in writing to participate in the research.

Statistical calculations were performed using the IBM SPSS 24 software. The characteristics of the researched sample were based on the calculation of the percentage distribution of the qualitative data frequen-

Table 2. Personality correlates of life on credit in a group of patients with osteoarthritis

Model	Measures of model fit			Regression weights for predictors				
	R^2*adjusted*	*F*	*p*	*B*	*SE*	*β*	*t*	*p*
Step 1								
Threat	0.09	5.99	0.018	0.03	0.01	0.33	2.45	0.018
Step 2								
Threat	0.16	5.73	0.006	0.06	0.02	0.63	3.37	0.001
Harm/loss				- 0.06	0.03	- 0.42	2.23	0.03

Source: Our own research results.

Table 3. Personality correlates of life on credit in a group of healthy people

Model	Measures of model fit			Regressionweights for predictors				
	R^2*adjusted*	*F*	*p*	*B*	*SE*	*β*	*t*	*p*
Step 1								
Impulsiveness	0.12	13.31	0.001	0.05	0.01	0.36	3.65	0.001
Step 2								
Impulsiveness	0.15	8.99	0.001	0.05	0.01	0.36	3.71	0.001
Self-esteem				0.02	0.01	0.20	2.05	0.044
Step 3								
Impulsiveness	0.19	8.13	0.001	0.03	0.01	0.25	2.35	0.021
Self-esteem				0.05	0.01	0.35	3.66	0.001
Threat				0.03	0.01	0.30	2.90	0.05
Step 4								
Impulsiveness	0.23	7.68	0.001	0.03	0.01	0.26	2.53	0.13
Self-esteem				0.05	0.01	0.36	3.91	0.001
Threat				0.04	0.01	0.38	3.52	0.001
Openness to experience and sense of humor				- 0.04	0.02	- 0.22	2.27	0.026
Step 5								
Impulsiveness	0.28	8.17	0.001	0.03	0.01	0.26	2.61	0.011
Self-esteem				0.04	0.01	0.34	3.76	0.001
Threat				0.04	0.01	0.38	3.72	0.001
Openness to experience and sense of humor				- 0.06	0.02	- 0.33	3.24	0.002
Empathy				- 0.04	0.01	- 0.27	2.79	0.007
Step 6								
Impulsiveness	0.31	7.81	0.001	0.03	0.01	0.30	2.30	0.004
Self-esteem				0.04	0.01	0.33	3.78	0.001
Threat				0.04	0.01	0.37	3.66	0.001
Openness to experience and sense of humor				- 0.06	0.02	- 0.34	3.44	0.001
Empathy				- 0.04	0.01	- 0.31	3.18	0.002
Need for socialapproval				0.07	0.03	0.19	2.09	0.039

Source: Our own research results.

cy, and descriptive statistics of quantitative variables. The predictive analytics was performed using multivariable stepwise regression. In the work, the boundary point of committing type I error is 0.05.

Results

In the group of people undergoing rehabilitation due to osteoarthritis, calculations made it possible to build a two-step model, in which two predictors explaining 16% of the variance of the explanatory variable (life on credit) were introduced.

In the first step, an appraisal of the stress situation related to the current economic situation as a threat was introduced as an explanatory variable. The model proved to fit the data well and explained 9.0% (R^2 *adjusted* = 0.09) of the variance of the dependent variable, $F(1, 49) = 5.99$; $p = 0.018$. In the second step, an appraisal of the stress situation related to the current economic situation as a harm / loss was introduced to the model. The model proved to fit the data and explained 16.0% (R^2*adjusted* = 0.16) of the variance of the explained variable, $F(2, 48) = 5.73$; $p = 0.006$.

The model developed reveals that in the group of people undergoing rehabilitation due to osteoarthritis, significant predictors of incurring financial liabilities are the appraisal of the stress situation related to the current economic situation as a threat, $\beta = 0.64$; $p = 0.001$ and perceiving it as harm or loss, $\beta = -0.42$; $p = 0.030$.

The relationship between the assessment of a stressful situation related to the current economic situation as a threat and the explained variable is directly proportional, and between the perception of a stressful situation as harm or loss and life on credit - negative, which implies that the more the current financial situation is treated as a potential threat for people diagnosed with osteoarthritis, the higher their inclination to incur financial liabilities. In turn, the higher the sense of harm or economic losses of the examined patients, the lower their readiness to take credit. The results of the predictive analysis in the group of patients with osteoarthritis are presented in Table 2.

In the healthy group, calculations made it possible to build a six-step model in which six predictors explaining 31% of the variance of the variable life on credit were introduced.

In the first step, impulsiveness was introduced as the explanatory variable. The model proved to fit the data and explained 12.0% (R^2*adjusted* = 0.12) of the variance of the dependent variable, $F(1, 49) = 13.31$; $p = 0.001$. In the second step, the self-assessment was introduced to the model. The model also proved to fit the data and explained 15.0% (R^2*adjusted* = 0.15) of the variance of the explained variable, $F(2, 48) = 8.99$;$p = 0.001$. In the third step, the appraisal of the stress situation related to the economic situation as a threat was introduced to the model. The model also proved to fit the data and explained 19.0% (R^2 *adjusted* = 0.19) of the variance of the dependent variable, $F(3, 47) = 8.13$; $p = 0.001$. In the fourth step, the model was supplemented by variable openness to new experiences and a sense of humor. The model also proved to fit the data and explained 23.0% (R^2 *adjusted* = 0.23) of the variance of the dependent variable, $F(4, 46) = 7.68$; $p = 0.001$. In the fifth step, empathy was introduced to the model. The model also proved to be correctly matched to the data and explained 28.0% (R^2*adjusted* = 0.28) of the variance of the explained variable, $F(5, 45) = 8.17$; $p = 0.001$. In the sixth step, the model was supplemented with a parameter illustrating the need for social approval. It proved to be correctly matched to the data and explained 31.0% (R^2 *adjusted*= 0.31) of the variance of the dependent variable, $F(4, 44) = 7.81$; $p = 0.001$.

The developed model reveals that in the healthy group significant predictors of life on credit are impulsivity, $\beta = 0.30$; $p = 0.004$, self-esteem, $\beta = 0.33$; $p = 0.001$, assessment of the stress situation related to the material situation as a threat, $\beta = 0.37$; $p = 0.001$, openness to new experiences and sense of humor, $\beta = -0.34$; $p = 0.001$, empathy, $\beta = -0.31$; $p = 0.002$ and the need for social approval, $\beta = 0.19$; $p = 0.039$.

The dependencies between impulsivity, self-esteem, regarding stress situation related to the economic situation as a threat and the need for social approval and the explained variable are directly proportional, while the relation between openness to new experiences and sense of humor and empathy and life on credit - negative, which indicates that together with the increase of emotional hyper-reactivity, negative anticipation of the material situation and the need to gain the acceptance of other people, the willingness to incur financial obligations increases. In turn, the inclination to indebtedness of healthy people decreases with the increase of motivation to undertake new tasks, the tendency to experience positive emotions and empathy. The results of the prediction analysis carried out in the healthy group are presented in Table 3.

Discussion

The results of the research indicate that for people representing the clinical group, perceiving a stressful situation as a threat is an important indicator of getting into debt. These people are above all concerned about upcoming difficulties that have not yet occurred. The possibility of experiencing harm and loss is paralyzing for them. This perception of upcoming events, perhaps the goal of avoiding them, is conducive to making decisions about taking loans. It is worth mentioning that such a perception of stressful situations is characteristic of people experiencing emotions of a depressive character, i.e.: feelings of depression, strong tension and anxiety, which are associated with high intensity of neuroticism.[16-18]

At the same time, the perception of stressful situations as the loss of what is valuable and important for a given person seems to be a factor protecting against incurring liabilities for persons belonging to a clinical group. In this case, however, these feelings are connected with the loss or harm experienced currently or earlier. When it comes to emotional states, they look the same as those in the situation of perceiving stressful situation as a threat (described above).[19]

For healthy people, the perception of stressful situations as a threat (which may appear) is also related to making decisions about debt. Additional determinants are: impulsiveness, high level of self-esteem and strong need for social approval. People who are self-confident willingly take credits and they have a strong need to appreciate their own personal qualities. At the same time, these people do not always have the ability to make a critical assessment of reality - concerning themselves and the situation, as well as the ability to realistically deal with the difficulties encountered. They are inclined to make hasty, often risky decisions and actions, not fully predicting their consequences and taking responsibility for them.[20,21]

In turn, people who are avoiding debts are characterized by low openness to experience, low sense of humor and low empathy. Therefore, these are people who definitely prefer predictability, steady and unchanging principles; they are reluctant to changes - especially sudden and spontaneous. These features give them a sense of safe functioning in their personal, family and professional lives. In relationships with others, they remain serious, have a large emotional distance, and are more focused on their own needs than on others.[22-24]

Thus, comparing the main correlates of "living on credit" in two researched groups, it can be stated that the fact of experiencing health difficulties makes the personality features and interpersonal relations irrelevant in this aspect. Life difficulties associated with experiencing stress, and in particular the way in which it is perceived, in this case become a leading factor.[25,26]

The research does not take into account the relationship between the time of incurring financial debt and the occurrence of the disease (before, during or after its occurrence). Nevertheless, it can be clearly indicated that the fear of experiencing loss is conducive to making decisions about indebtedness regardless of the current or previous experience of the disease (the main factor in each group). In emergency situations, the desire to avoid the experience of impending loss (harm) makes subjects ready to make far more risky decisions, regardless of the personality predispositions. On the other hand, the real experience of loss or such a perception of a stressful situation is a factor that causes greater caution before undertaking the risk of indebtedness.[27]

This undoubtedly indicates the necessity of support and reliable advice for people in the situation of the disease who are struggling with financial difficulties and face the dilemma of incurring financial liabilities. Research results indicate that in such a situation, the level of desperation of potential borrowers can be very intense, and the desire to avoid losses may contribute to excessive hopes in persons or institutions offering financial assistance.[28,29]

Conclusion

1. The analysis showed that particular personality traits are linked to incurring financial liabilities, and health condition is the moderator of the relationships.
2. In the group of patients diagnosed with osteoarthritis, the factor conducive to living on credit is the assessment of a stressful situation related to the current economic situation seen as a threat, and perceiving it as a harm or loss makes people refrain from incurring financial liabilities.
3. In the healthy group, impulsivity, self-esteem, the appraisal of stress situation related to the economic situation as threats and the need for social approval are the factors that contribute to indebtedness, and openness to new experiences, sense of humor as well as empathy play a protective role against making decisions about incurring financial liabilities.
4. The results indicate the need to take into account the specificity of the health situation of participants of psycho-preventive programs aimed at helping people who seek support when making decisions about incurring financial liabilities.

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ORIGINAL PAPER

Agnieszka Jankowicz-Szymańska [1 (ABCDEF)], Katarzyna Wódka [1 (BCDF)],
Edyta Mikołajczyk [2 (FG)]

Is valgus foot always flat? The longitudinal arch of the foot and hindfoot valgus in 10–12 year-olds

[1] Institute of Health Sciences, State Higher Vocational School, Tarnow, Poland
[2] Department of Physiotherapy, University of Physical Education, Krakow, Poland

ABSTRACT
Introduction. Flat foot is still a controversial topic. As of yet, there are no unified definitions of this deformation.
Aim. To assess the correlation between the longitudinal arch of the foot and hindfoot valgus in school-age children.
Material and methods. A total of 362 pupils, 183 girls aged 10.96 ± 0.78 yrs and 179 boys aged 10.89 ± 0.78 yrs, participated in the study. Their height and weight were measured and their Body Mass Index (BMI) weight status was categorized. The Arch Index (AI) was used to assess the longitudinal arch of the foot. Hindfoot valgus was measured by a goniometer and defined as the angular deviation between the tibial anatomical axis and the calcaneus longitudinal axis.
Results. About 1/3 of the participants had hollow feet and about a fifth of them had flat feet. No correlations between the longitudinal arch of the foot and the hindfoot valgus were discovered; however, a correlation between excessive weight and the longitudinal flat foot was revealed. No correlations between BMI and hindfoot malalignment were found.
Conclusion. Hindfoot valgus was prevalent in a considerable proportion of boys and girls with the flat, normal and hollow foot. Therapeutic correction of valgus feet should be varied and should depend on the quality of the longitudinal arch of the foot.
Keywords. flat foot, hindfoot valgus, school children

Introduction

The ability to maintain good posture is not inborn. Children, acquiring new locomotor skills, gradually manage control over their body and develop their postural pattern. The lower limb alignment undergoes especially dynamic development. In infants and toddlers, flat feet are a common and normal condition until about age ten.[1,2] During that time, due to the impact of negative external or internal stimuli, foot deformities may develop. Observations of the relationship between the status of body mass and the quality of the arching of the feet indicate a greater incidence of flat feet in children with excessive body mass.[3] Knees valgus are also associated with incorrect foot shape.[4] Foot deformities are very common. Most pediatric orthopedic consultations concern flat feet.[5]

From the point of view of biomechanics, flat feet are a complex deformity. They are caused by ligamentous laxity and lack of muscle control.[6] This study aimed to assess correlations between the longitudinal arch of

Corresponding author: Agnieszka Jankowicz-Szymańska, e-mail: jankowiczszymanska@gmail.com

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

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the foot and hindfoot alignment in children aged 10-12 years. It was investigated which of the above-mentioned deformities prevailed more often and whether excessive weight increased the risk of their development.

Materials and methods

The study was conducted in September and October 2015. Prior to the start of the study, consent of the local ethics committee was obtained (no 3/0177/2014). Four schools were randomly selected and all pupils attending grades four through six were initially invited to participate in the study. Participants were assigned to the study samples based on a written informed consent of their parent or a legal guardian and lack of medical certification of disability. Those children, who had previously been treated due to hip dysplasia or Perthes disease, and those who had suffered from injuries to their lower limbs within six months prior to the study, were excluded from the examination. Eventually, a total of 362 pupils aged 10-12 years (10.89 ± 0.78), including 183 girls (50.5%) and 179 boys (49.5%), participated in the study.

Participants' height and weight measurements were taken, their BMI calculated and their weight status categorized. Body height was measured within 1 cm by means of a calibrated anthropometer (ZPH Alumet No 010208, Warsaw, Poland), measuring the distance from the floor to the highest point on the head. During measurements, participants stood motionless with their legs together and feet flat on the floor and with their sight directed straight ahead. Body weight was measured within 0.1 kg on the Tanita scales (bf-350 Tanita Corporation of America, Inc., Arlington Heights, Illinois, USA). Measurements were taken from participants undressed to their underwear and without footwear. Based on the results obtained, BMI was calculated dividing body weight (kg) by the square of body height (m). Participants' body weight status was categorized using the BMI-for-age thresholds for overweight and obesity, developed for children by Cole et al.[7]

The baroresistive BTS P-walk platform (**BTS Bioengineering Corp., NY, USA**) was used to measure the longitudinal arch of the foot. The Arch Index (AI) was determined in two feet standing, for the right and left foot, calculated as the ratio of the area of the middle third of the footprint to the entire footprint area. The AI is an easy and objective method of assessing the quality of the longitudinal arch of the foot, and its characteristic value for the foot with a normal arch equals from 21% to 28%. Its values below 21% refer to a high foot arch, and those above 28% denote flat foot. The hindfoot alignment was measured by a goniometer (KaWe, Horn Wellness Group Ltd. Poznan, Poland) and defined as the angular deviation between the tibial anatomical axis and the calcaneus longitudinal axis. The angle above 5° indicated hindfoot valgus.[8] Participants assumed relaxed posture and stood with their legs a little apart. Individual measurements were taken in a pre-arranged order, by the same experienced physiotherapists, before the noon and in a well sun-filled room.

The mean, median, minimum and maximum values and standard deviation were used to analyze the data collated. The normal distribution of variables was assessed by means of the Shapiro-Wilk test. The difference between the samples was determined by means of the t-test for independent samples (at normal distribution), and by the U Mann-Whitney test (at lack of normal distribution) or by the Kruskal-Wallis and post hoc Tukey's tests (at comparing three samples at the same time). Correlations between variables were assessed by Pearson's linear correlation. The level of significance was accepted at $p = 0.05$.

Results

Girls were slightly taller and lighter than boys. BMI did not significantly differentiate boys and girls (Table 1). Excessive body weight was discovered in 99 participants, out of which 72 were overweight (18.9%) and 27 obese (7.5%). Overweight was more typical of girls, and obesity in boys (Table 2). The AI for the right and left foot was slightly greater in boys, which implied that they had lower foot arches than girls (Table 1). A fallen arch in the right foot was found in 19.1% of participants, i.e. in every fourth boy and in every sixth girl. A fallen arch in the left foot was discovered in 16.3% of participants, i.e. in every fourth boy and every ninth girl (Table 2). A high arch in the right foot was found in 31.5%, and in the left foot in 36.7% of all participants. In the case of both feet, high foot arches were more typical of girls.

The hindfoot valgus angle in the right foot was greater in girls, and in the left foot in boys; however, those differences were not of statistical significance (Table 1). The hindfoot malalignment was revealed in over 50% of participants. The valgus deformity in the right foot prevailed more often in girls, while in the left foot in boys (Table 2). The comparison of the hindfoot malalignment in participants with the high, normal and flat foot disclosed a statistically significant difference only in the size of the hindfoot valgus angle in the right foot in children with the normal foot and flat foot (Table 3). The valgus deformity in the right foot was discovered in 207 participants - 31.4% of them had the high-arched foot, 45.4% normal and 23.2% flat foot. The valgus deformity in the left foot was found also in 207 participants - 34.8% of them had the high-arched, 45.9% normal and 19.3% flat foot.

The lowering of the longitudinal arch of the foot correlated with increased BMI (Table 3). Significant differences in the BMI value were seen between participants with the high-arched and normal foot, and between the high-arched and flat foot. The analysis of dependencies

Table 1. Comparison of variables in girls and boys

Variable	Group	Mean	Median	Minimum	Maximum	Stand. Dev.	p
Height [cm]	Girls	147.88	147.80	128.40	171.00	9.55	0.34[a]
	Boys	147.11	145.90	124.40	178.30	8.95	
Weight [kg]	Girls	41.68	41.80	21.00	73.20	10.67	0.34[b]
	Boys	41.70	38.40	23.60	100.60	12.98	
BMI [kg/m^2]	Girls	18.80	18.59	12.70	29.32	3.27	0.51[b]
	Boys	18.97	17.74	11.85	38.57	4.26	
AI right foot [%]	Girls	22.06	23.46	1.96	36.42	7.03	0.08[b]
	Boys	22.90	24.80	0.90	41.64	8.36	
AI left foot [%]	Girls	20.95	22.37	1.90	37.28	7.22	0.09[b]
	Boys	22.17	23.38	0.78	38.66	8.12	
Right hind valgus angle [°]	Girls	6.32	6.00	0.00	13.00	2.65	0.75[b]
	Boys	6.25	6.00	2.00	15.00	2.49	
Left hind valgus angle [°]	Girls	5.96	6.00	0.00	13.00	2.53	0.75[b]
	Boys	6.71	7.00	1.00	18.00	2.81	

*statistically significant differences, [a] t test, [b] U Mann-Whitney test

Table 2. Weight status, foot arches and hindfoot alignment in girls and boys

Variable	Group	Girls		Boys		All	
		n	(column %)	N	(column %)	n	(column %)
Weight status	Normal	138	(75.4%)	125	(69,9%)	263	(72.6%)
	Overweight	38	(20.8%)	34	(19.0%)	72	(18.9%)
	Obesity	7	(3.8%)	20	(11.1%)	27	(7.5%)
Right foot arch	High arched	61	(33.3%)	53	(29.6%)	114	(31.5%)
	Normal	94	(51.4%)	85	(47.5%)	179	(49.4%)
	Flat	28	(15.3%)	41	(22.9%)	69	(19.1%)
Left foot arch	High arched	73	(39.9%)	60	(33.5%)	133	(36.7%)
	Normal	90	(49.2%)	80	(44.7%)	170	(47.0%)
	Flat	20	(10.9%)	39	(21.8%)	59	(16.3%)
Right hindfoot alignment	Normal	76	(41.5%)	78	(43.6%)	154	(42.5%)
	Valgus	107	(58.5%)	101	(56.4%)	208	(57.5%)
Left hindfoot alignment	Normal	87	(47.5%)	67	(37.4%)	154	(42.5%)
	Valgus	96	(52.5%)	112	(62.6%)	208	(57.5%)

Table 3. Comparison of variables in children with high-arched, normal and flatfoot (Kruskal-Wallis and post hoc Tukey's tests)

	Variable	Foot arching groups	Mean	Median	Minimum	Maximum	Stand. Dev.	p
Right foot	Hindfoot alignment [°]	High-arched	6.39	7.00	0.00	13.00	2.65	H & N p=0.38
		Normal	6.91	7.00	2.00	15.00	2.50	H & F p=0.55
		Flatfoot	6.91	7.00	2.00	15.00	2.50	N & F p=0.028*
	BMI [kg/m^2]	High-arched	17.42	16.64	11.85	24.62	2.98	H & N p=0.00002*
		Normal	19.34	18.88	13.10	31.75	3.70	H & F p=0.00003*
		Flatfoot	20.05	19.24	13.13	38.57	4.45	N & F p=0.99
Left foot	Hindfoot alignment [°]	High-arched	6.26	6.00	0.00	14.00	2.73	H & N p=0.99
		Normal	6.27	6.00	1.00	18.00	2.74	H & F p=0.67
		Flatfoot	6.68	7.00	2.00	12.00	2.50	N & F p=0.64
	BMI [kg/m^2]	High-arched	17.38	16.79	11.85	25.55	2.88	H & N p=0.00004*
		Normal	19.39	18.79	13.10	31.75	3.70	H & F p=0.0000001*
		Flatfoot	20.71	20.27	12.98	38.57	4.57	N & F p=0.17

* statistically significant differences, H= high-arched foot, N=normal arched foot, F=flatfoot

Table 4. Correlations between variables

Correlated variables	Right foot		Left foot	
	r	p	R	p
BMI & Arch Index	0.31	0.0001*	0.32	0.0001*
BMI & Hindfoot valgus angle	-0.07	0.19	-0.09	0.06
Arch Index & Hindfoot valgus alignment	0.23	0.06	-0.01	0.85

*statistically significant correlation

between variables also revealed significant positive correlation between the BMI and AI values for the right and left foot (Table 4). No significant correlations between the AI and the hindfoot valgus angle or between BMI and the hindfoot valgus angle were discovered.

Discussion

The process of shaping foot arches completes at about age ten when the foot assumes its mature structure.[9] Foot deformities observed at that time may become a chronic, life-long condition. Our study revealed foot malalignment in more than 50% of the 10-12-year–old participants. A fallen longitudinal arch in the right foot was typical of every sixth, and in the left foot, of every fifth participant. One third of our participants had high foot arches. The hindfoot valgus deformity was seen in 57.5% of all participants. A similar prevalence of flat foot was observed in the 9-year-olds by El et al.[10], who revealed moderately or considerably fallen arches in 17.2% of their participants. Flat feet and plano-valgus feet were also disclosed in 16.7% of the 10-12-year-olds in the study of Szczepanowska-Wołowiec et al.[11]

Flat feet have been a subject matter of numerous academic and research discussions. On the one hand, some researchers claim that pediatric fallen foot arches are asymptomatic and they do not require any treatment.[12] On the other hand, other researchers maintain that flat feet lead to unfavorable changes in the dynamic functions of the whole lower limb and increase the incidence of knee, hip and back complaints.[13,14] According to the most common definition of flat feet, this deformity consists of fallen foot arches, forefoot abduction and the hindfoot valgus deformity.[5,15-17] Hindfoot valgus concomitant with flat foot was confirmed by Coughlin and Kaz.[18] It should also be mentioned that the abduction of the calcaneus results in changes to the gait pattern connected with the rotation in the hip joint and the pelvic tilt in the sagittal plane.[16]

Flat feet are more typical of boys than of girls. It was confirmed in the studies of Changa et al. and Ezema et al., and also by our findings.[19,20] The correlation of the hindfoot valgus deformity with gender is not so distinct. In our study, the valgus deformity in the right foot was slightly more often discovered in girls, while in the left foot more frequently in boys. In the right foot, hindfoot valgus was found in 57% of participants with the high-arched foot, in 53% with normal and in 71% with flat foot. In the left foot, those values were 55%, 57% and 68% respectively. No significant differences in the value of the hindfoot valgus angle in children with the high-arched, normal and flat foot were found despite a greater prevalence of the hindfoot valgus deformity in children with flat feet. No significant correlations between the hindfoot valgus angle and the height of the medial longitudinal arch were also found by Kanatli et al.[21] In their study, they examined 206 voluntary participants aged 4-20 years, in which the mean hindfoot valgus angle for the whole sample was $5.2^{o} \pm 3.3$. That score was lower than in our study.

One should take into account a considerable incidence of high foot arches in the participants in our study. They prevailed, as mentioned before, in 57.5% of all participants. In the 4-13-year-olds examined by Woźniacka et al., that proportion was even higher and it reached above 61%.[22] In their study, the hollow foot was also discovered in obese children, although the lowered medial longitudinal arch of the foot was more typical of their participants. In our study, in the sample of obese children, high foot arches were disclosed in the right foot in 3.7%, and in the left foot in 11.1% of participants. A significant correlation between excessive body weight and flat feet was observed as well. That correlation was also confirmed by Halabchi et al.[12] Moreover, Ezema et al. reported that obese school children had flat feet three and a half times more often than their mates with healthy body weight.[20] Pauk et al. claimed that the 8-14-year-olds with a low BMI less often had the plano-valgus foot.[23] According to the reports on young adolescents of both genders, both fallen foot arches and hindfoot valgus more often prevailed in obese individuals.[24] In our study, a correlation between BMI and the height of the medial longitudinal arch of the foot was found but no significant correlation either between BMI and the hindfoot valgus angle, or between the height of the medial longitudinal arch of the foot and the hindfoot valgus angle were revealed.

Conclusions

1. The hindfoot valgus deformity prevailed in school children more frequently than flat feet. It was manifested by both girls and boys with the flat, normal and hollow foot.
2. The valgus foot deformity did not correlate with the medial longitudinal arch of the foot. Therapeutic corrections of the valgus deformity of the foot should be varied, depending on the quality of the longitudinal arch of the foot.
3. Although the valgus foot deformity did not correlate with excessive body weight, there was a significant correlation between the BMI and the longitudinal arch of the foot.

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Patrycja Pawlik (ABCDEF), Anna Stanek (ABCDEF),
Marzena Wyganowska-Świątkowska (FG), Katarzyna Błochowiak (ACF)

The epidemiological pattern of oroantral communication – a retrospective study

Department of Oral Surgery and Periodontology, Karol Marcinkowski, Medical University in Poznan, Poznan, Poland

ABSTRACT

Introduction. Oroantral communication (OAC) between the maxillary sinus and the oral cavity is an infrequent post-surgical complication occurring most commonly after extraction of posterior maxillary teeth.

Aim. To present the characteristics of OAC and predisposing factors as well as evaluate postoperative pharmacological therapy and complications in patients with an OAC.

Material and methods. In this retrospective study, medical records of 63 patients with diagnosed OAC between 2011 and 2018 were analyzed.

Results. The most frequent causes for tooth extraction leading to an OAC were periodontitis (n=34; 54%), carious destruction of the tooth (n=14; 22.2%), and tooth impaction (n=10; 16%). First molars (n=28; 44.4%), second molars (n=14; 22.2%) and third molars (n= 13; 20.6%) were the most frequently related teeth to OACs. The majority of OACs appeared in the fourth (n=22; 35%) and third (n=20; 31.7%) decades of life.

Conclusion. OACs are rarely seen on an everyday basis by general practitioners; however, if left untreated, they may lead to further serious complications. Proper postoperative precautions must be taken in order to prevent further complications, and thus the evaluation of predisposing factors is of great importance.

Keywords. maxillary sinus, oroantral communication, tooth extraction

Introduction

Oroantral communication (OAC) is a rare surgical complication that occurs when an opening is created between the maxillary sinus and the oral cavity. If left untreated, an OAC can lead to further complications, such as the formation of an oroantral fistula (OAF) or an infection of the maxillary sinus.[1] There are a variety of factors that may cause or lead to an OAC, including trauma, tumors, cysts and other pathological entities. Nevertheless, the most frequent cause of OACs is the extraction of maxillary posterior teeth, which can be the result of a close association of maxillary premolars and molars root apices to the maxillary sinus floor.[1-3] This relationship between the root apices and the maxillary si-

Corresponding author: Patrycja Pawlik, e-mail: patrycja.pawlik91@gmail.com

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nus floor makes it much easier for complications such as an OAC to occur during an extraction. The occurrence of OACs varies depending on the maxillary tooth being extracted.[2,4,5] OACs may be an iatrogenic complication associated with inadequate surgical technique and therefore it is important to have a proper preoperative plan as well as select the appropriate procedure in the case of maxillary posterior tooth extraction. Such procedures may include preoperative radiographs to assess the location of the roots and state of the maxillary sinus as well as a change in the surgical technique used. Management of OACs is predominantly dependent on the size of the communication between the maxillary sinus and the oral cavity, as well as the overall health status of the patient. In patients with healthy sinuses and an OAC less than 5 mm in size, the communication tends to close spontaneously after the development of a blood clot in the socket. If the communication is between 2 and 6 mm, management includes the placement of a collagen plug into the socket and figure-of-eight sutures. In cases where OACs are larger in size, surgical intervention is required to close the opening.[6,7] Once patients undergo surgical intervention and OAC closure, it is important to follow postoperative sinus precautions to avoid further complications, such as sinusitis or the formation of an OAF. Studies show that if an OAC is maintained open for 48 hours or more, chronic inflammation of the sinus membrane may occur and permanent epithelization of the buccosinusal fistula may form, greatly increasing the risk of sinusitis.[8] The most important issue is to quickly and correctly diagnose OACs to counteract these complications and choose the best method of treatment. Postoperative precautions include the use of decongestants and antibiotics such as penicillin or clindamycin for 7 to 10 days.[9] It seems reasonable to develop a process for the extraction of posterior maxillary teeth, which will minimize the risk and incidence of OAC and associated complications.

Aim

The aim of this retrospective study is to present the characteristics of OAC and predisposing factors as well as evaluate postoperative pharmacological therapy and complications in patients with an OAC.

Material and methods

For this retrospective study, the medical records of 82 patients with documented diagnosis of OAC who were treated between January 2011 and March 2018 at the Department of Oral Surgery and Periodontology and the Emergency Department, Poznan University of Medical Sciences, Poland were reviewed. Initially, this study included 82 patients, however, eighteen patients were excluded from further analysis due to incomplete data. The final study sample included 63 patients, including 33 women (52.4%) and 30 men (47.6%) aged 15 -91 (mean age: 40). In all cases, OAC was diagnosed using the nose-blowing test right after tooth extraction. The comprehensive dental and medical history was taken from all patients in the Department of Oral Surgery and Periodontology and the Emergency Department, Poznan University of Medical Sciences, Poland. The data recorded for each patient included sex, age, tooth number, right or left side involvement, reason for tooth extraction, anesthetic used, prescribed postoperative antibiotics, supplementary drugs, and short-term complications. Anesthesia was performed before the surgical procedure and a buccal flap or a Wassmund flap with Borusiewicz modification was performed for OAC closure. Sutures were removed 6-10 days postoperatively. Antibiotics, either clindamycin or amoxicillin were prescribed to patients postoperatively, as well as nasal decongestants and nonsteroidal anti-inflammatory drugs (NSAIDs). The patients were instructed to avoid nose blowing and sneezing with a closed mouth for 2 weeks, strenuous physical activities, smoking, use of straw as well as rolling their tongue over the wound with sutures.

This data was then analyzed and evaluated using descriptive statistical methods using IBM SPSS Statistics software (v. 23.0, Chicago, IL). P values lower than 0.05 were considered as statistically significant.

Before any study procedure was carried out, written informed consent was obtained from every subject. The study was carried out in accordance with the ethical standards set by the World Association Declaration of Helsinki.

Results

The most common diagnosis for tooth extraction that later led to an OAC was periodontitis (54%), followed by carious destruction of the tooth (22.2%), impaction of third molars (16%) and chronic OAC from previous extractions (7.9%). The distribution of diagnosis for tooth extraction was demonstrated in Table 1. There was a statistically significant difference between the diagnosis for tooth extraction and the age of the patient (p=0.007). A significant correlation was found between the diagnosis for extraction and the tooth number (p<0.05).

The majority of OACs appeared in the fourth and third decades of life (Table 2). The teeth related to the highest occurrence of OAC were the first molars (44.4%), followed by the second molars (22.2%) and third molars (20.6%) (Table 3). A statistically significant difference was observed between the age of the patient and the extracted tooth (p=0.007). In addition, statistical significance was reported between the third molars and the canine (p=0.036), as well as the third molars and the second molars (p=0.019). Conversely, there was no statistical significance between the age of the patient and the postoperative complications.

Table 1. Distribution of diagnosis for tooth extraction that later led to an OAC

Diagnosis	Frequency (n)	Percentage (%)
Periodontitis		
– Acute	8	12.7
– Chronic	26	41.3
Caries	14	22.2
Impaction of third molars	10	15.9
OAC from previous extraction	5	7.9
Total (n)	63	100

Table 2. Distribution of patients with OAC according to decade of life and tooth

Age group	Canine	First premolar	Second premolar	First molar	Second molar	Third molar	Total number of OACs
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
21 – 30	0 (0.0)	2 (3.1)	1 (1.6)	8 (12.7)	1 (1.6)	8 (12.7)	20 (31.7)
31 – 40	0 (0.0)	1 (1.6)	0 (0.0)	11 (17.5)	7 (11.1)	3 (4.8)	22 (35.0)
41 – 50	0 (0.0)	0 (0.0)	1 (1.6)	4 (6.5)	0 (0.0)	1 (1.6)	6 (9.7)
51 – 60	0 (0.0)	1 (1.6)	0 (0.0)	1 (1.6)	2 (3.1)	1 (1.6)	5 (7.9)
61 – 70	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.1)	3 (4.8)	0 (0.0)	5 (7.9)
71 – 80	1 (1.6)	0 (0.0)	0 (0.0)	1 (1.6)	1 (1.6)	0 (0.0)	3 (4.8)
81 – 90	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.6)
91 – 100	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.6)	0 (0.0)	0 (0.0)	1 (1.6)

Frequency (n). *P* value =0.007.

Table 3. Incidence of OACs related to tooth and side involvement

Tooth	Right		Left	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
Canine	2	3.2	0	0.0
First premolar	3	4.8	1	1.6
Second premolar	2	3.2	0	0.0
First molar	14	22.2	14	22.2
Second molar	10	15.9	4	6.3
Third molar	7	11.1	6	9.5
Total (n)	38	60.4	25	39.6

Table 4. Incidence of postoperative complications

Complication	Frequency (n)	Percentage (%)
Trismus	7	11.11
Fever	2	3.17
Pain	9	14.29
Swelling	9	14.29
Total (n)	27	42.86

The right side of the face was more commonly involved with OAC (Table 3). Additionally, no correlation was observed between the sex of the patient and the diagnosis for tooth extraction, as well as between the sex of the patient and tooth number. The occurrence of OAC was higher in females (52.4%) than in males (47.6%). No statistically significant difference was seen between the sex of the patient and postoperative complications.

Clindamycin was prescribed to 70% of patients, and the remaining 30% received amoxicillin. Nasal decongestants were prescribed in most cases (73%), as well as NSAIDs (60%). The anesthetic most commonly used was articaine 4% with 1:200 000 epinephrine (71.43%), followed by mepivacaine 3% (27%). There was a statistically significant difference between the age of the patient and the anesthetic used ($p<0.05$). A statistically significant correlation was observed between the diagnosis given for tooth extraction and the postoperative use of nasal decongestants ($p=0.012$). Postoperative complications were observed in 42.86% of cases (Table 4). Though, there was no statistically significant difference

between postoperative complications and tooth number, nor between postoperative complications and age of the patient. There was a lack of statistical significance between the use of antibiotics and the incidence of postoperative complications. Moreover, OAF and maxillary sinusitis were not documented complications in follow-up appointments. No significant correlation was observed between the age of the patient and the antibiotics prescribed.

Discussion

OAC is considered a rare complication of oral surgery, with studies reporting various incidence rates, ranging from 0.31% to 13%, and with the occurrence of OAC differing depending on the type of tooth being removed.[2,5,10,11] In the present study, the most common causes of tooth extraction that led to OACs were periodontitis, carious destruction of the tooth, and impaction. Punwutikorn et al., also observed these diagnoses for extractions that led to OAC, but they were less common than OACs caused by dentoalveolar abscess.[10] We observed a significant difference between the diagnosis of periodontitis and impaction. The high frequency of OAC due to periodontitis may be associated with the presence of periapical lesions and narrowing of the periodontal space, which could result in a more difficult extraction and higher risk of OAC.[12] Impaction is most commonly seen in third molars, and are therefore increasingly more difficult to extract.[9] Expansion of the sinus is usually completed after the eruption of permanent teeth, but occasionally, the sinus is further pneumatized, particularly after the removal of one or more posterior maxillary teeth, to occupy the remaining alveolar process.[9] However, our results show that the maxillary first molar was the most important causative tooth of OAC. We concur with various authors who assert extraction of the first molar most often leads to such pathology.[10,13,14] As molar extractions were only observed in 20.6% of cases, our results are in contrast to Wachter et al., who attributed OAC being the most common complication in the maxilla due to the close proximity of the third molars to the maxillary sinus.[15] The main causative teeth of OAC are the last four maxillary, but most often tooth involvement is variable and depends on the consulted sample.[11,16-19]

There is no consensus among various authors concerning the frequency of left side or right side involvement. OAC was found to be as frequent on the left side as on the right by both Del Rey et al. and Punwutikorn et al,.[3,10] The current study showed 60.3% of OACs occurred during extractions performed on the right side of the face. However, Jones et al., found that roots were displaced into the left antrum more frequently than the right antrum.[20] Different degrees of pneumatization may be due to developmental variations during tooth eruption, which could lead to asymmetrical development of the maxillary sinuses, or as stated earlier, due to the removal of one or more posterior maxillary teeth.[9,21] Additionally, previous infections of the sinuses and upper respiratory tract may disturb or change sinus development or cause anatomical anomalies, for example from nasal septum deviation.[22] Hypertrophy of the nasal turbinates and tonsils could also interfere with symmetrical development of the sinuses.[21] Finally, surgical ergonomics will play a role in the performance of an extraction. The position of the dentist will affect the amount of force applied during the extraction, which can vary depending on their position for the side involved, as is determined by their preference to extract in a sitting or standing position, and left or right hand dominance.[9] Other factors, such as how wide the patient can open their mouth, the resiliency of the cheeks, perioral musculature, exaggerated gag reflex, airway liability, and overall patient cooperation may affect the overall performance of the extraction.[9]

Additionally, the literature shows conflicting results with respect to the variable of age and the occurrence of OAC. The risk of OAC increases after the third decade of life, which marks the completion of maxillary sinus development and thus the greatest size.[10] The risk of an OAC occurring in children is minimal due to the smaller size of the maxillary sinus.[3,10,13,16] The age of patients in our study ranged from 21 to 91 years, with a predilection for the group of 30-40 years of age, and a mean of 40 years old. The fourth and third decade of life showed the most frequent occurrences of OAC. Comparably, other authors observe a greater number of OAC in the third, fourth and fifth decades of life.[8,11,13,16,23] Guven justified the increased incidence in the fourth decade of life to the pneumatization of the maxillary sinus from loss of posterior maxillary teeth.[13] However, Del Rey et al., based their study on the appearance of OAC in the extraction of the third molar, and obtained an average of 21 years of age.[3] This is similar in respect to our results, which showed that OACs after third molar extractions occurred most often within the age group of 21-30 years. In a study by Abuabara et al., the average age obtained was 31.2 years old, basing this finding on the overall higher incidence of third molar extractions.[18] Conversely, Punwutikorn et al., found the greatest incidence of OAC occurred as of the sixth decade of life.[10] A higher risk of OAC may result from previous loss of adjacent teeth and increased pneumatization of the sinus, as well as sclerosis of the bone in the elderly, or periodontitis, which makes extraction difficult.[9,12]

In this study, a slight predominance of OAC was found among females. This finding is not in agreement with those reported by other studies, who attributed a greater frequency among males due to a more frequent indication of third molar extraction and increased tech-

nical difficulties than in women.[2,13] With respect to this variable, other studies do not report a difference.[8,3,10,16]

There is no clear decision among authors with respect to antibiotics prescription in patients who are diagnosed with OAC. The present study showed all 63 patients were prescribed antibiotics, with Clindamycin 0,6 given to 44 patients (70%). Nasal decongestants, NSAIDs, or a combination of both were also prescribed to patients. Studies show nasal decongestants are used because they facilitate shrinking of the nasal mucosa, keeping the antral opening patent, and NSAIDs are prescribed for pain control.[1,24] In patients without confirmed penicillin allergy, it is recommended to prescribe amoxicillin with or without clavulanic acid due to better absorption and reduced risk of side effects.[25] However, due to an increased risk of antibiotic resistance, such prescriptions given by dentists should be limited to certain oral bacterial infections with signs of spreading or systemic involvement, or to febrile or immunocompromised patients.[26-29] In our study, the justification for antibiotic prescription was not reported by dental practitioners in patient medical records. It has been shown that generally 30-50% of prescribed antibiotics are unnecessarily or not optimally prescribed.[30] Several authors agree on the recommendation of antibiotic prophylaxis in the postoperative treatment of OACs.[8,13,31] However, depending on the country, antibiotics are not always prescribed after simple extraction with OACs. For example, in the Netherlands, antibiotics and decongestants are only recommended in OACs existing longer than 24 hours or in OACs with evident non-purulent antral infection.[25] Our results showed that there was no significant difference between the drugs used, which leads us to believe that surgical technique is the most important factor contributing to OAC formation.

The present study showed 15.9% of patients reported symptoms of pain, swelling, trismus, and fever after OAC closure procedure. Such symptoms are known possible adverse effects of tooth extraction. We attribute these results to the extraction procedure itself rather than to the postoperative complications of OAC.[9] OACs cause microbial contamination from the oral cavity to the maxillary sinus.[16] If left untreated, an OAC may lead to further complications, including OAF formation, which can further lead to maxillary sinusitis. Previous studies show a sinusitis rate of 60% after the fourth day post-OAC, while others observed a sinusitis rate of 50% by the third day after the occurrence of OAC.[1] The need to confirm diagnosis of OAC within 24 hours is of great importance to ensure successful treatment, and thus eliminating the risk of further complications, such as maxillary sinusitis, from arising.

Infiltration anesthesia was most frequently performed using 4% articaine with 1:200 000 epinephrine, followed by 3% mepivacaine hydrochloride without epinephrine. There are several known benefits of vasoconstrictor addition to local anesthesia, including: reduced peak plasma concentration of the pharmacological agent, increased quality of anesthesia, prolonged duration, reduction of the minimum concentration of anesthetic required, and decreased blood loss during surgical procedures.[32] Articaine is considered advantageous because of its low toxicity, and ability to achieve excellent bone penetration.[33] There was statistical significance observed between the type of anesthesia used versus the age of the patient. We attribute the use of mepivacaine without epinephrine to the fact that with increasing age there is greater likelihood patients will present with existing chronic conditions, which could be contraindications for the use of vasoconstrictor. Such contraindicated conditions may include cardiovascular diseases, stroke, and hyperthyroidism, to name a few.[34]

Our study is the first Polish epidemiological description of OAC incidence during simple tooth extraction. One of the limitations of this study includes a small sample size. It would be beneficial to compare our research with other Polish medical centers to establish specific Polish epidemiological and population features. Other limitations include a lack of evaluation of the difficulty of each tooth extraction, a lack of longer postoperative control of patients, as well as a lack of radiographic control of the affected maxillary sinuses. Furthermore, there was limited general medical data available regarding sinus and respiratory tract disorders. Our study could not take into account the reason for the antibiotic prescriptions given to patients. This shows the importance of the need to further standardize these practices, as we emphasize the need for establishing protocols to prevent the occurrence of OAC.

Conclusion

This study attempted to determine the incidence of OACs that occurred after simple tooth extraction in a Polish general population. Extraction of a posterior maxillary tooth can be a difficult task even for the most experienced dentist and therefore the surgical techniques as well as the dentist's surgical skills are of great importance when it comes to avoiding postoperative complications such as OACs. Since the extraction of maxillary first, second and third molars most commonly leads to an OAC, it is essential to provide dentists with a proper guide for maxillary extractions and postoperative management. Imperative measures that could be useful in the prevention of OAC during routine extractions include proper radiological evaluation before routine extractions, especially in cases of diagnosed periodontitis and tooth impaction in the predisposed age group, ensuring medical history is taken of previous

sinus conditions, and adapting to appropriate surgical techniques after radiological evaluation. Furthermore, prevention of OAC which lead to further complications can be achieved by rapid and appropriate diagnosis after each extraction performed in the posterior region, and following proper postoperative precautions. These factors combined may greatly improve treatment success.

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ORIGINAL PAPER

Agnieszka Wybraniec [1(ABDF)], Miłosz Boguszewski [2(ABDF)], Norbert Nizio [2(ABDF)], Justyna Wyszyńska [1(DEFG)], Justyna Drzał-Grabiec [1(DEFG)]

Selected factors influencing physical fitness in the elderly

[1] Medical Faculty, University of Rzeszów, Rzeszów, Poland
[2] Faculty of Tourism and Health, Academy of Physical Education Józef Piłsudski in Warsaw, branch in Biała Podlaska, Biała Podlaska, Poland

ABSTRACT

Introduction. In effect of the ageing process, there are involutional changes in older adults both in the mental and in the physical spheres. Some factors may slow down the ageing process. Physical activity is one of these factors. Physical ability is one of the most important indicators of quality of life of older adults, and it can be shaped by physical activity.

Aim. The aim of the study was to assess chosen factors that influence physical ability of adults older than 65 years.

Material and methods. The study involved 63 older adults. The criterion for inclusion in the study was the age - older than 65 years. There were 37 women and 26 men in the study population. All the subjects were able to walk without assistance, and they had no contraindications to do functional tests. We used a questionnaire of our own design, three SPPB trials, Up&Go test; we calculated the BMI indices and assessed depression scale.

Results. We found that older adults rarely engage in physical activity. The low level of physical activity of the older adults was reflected in equally low physical ability level, as assessed by functional tests. We found a directly proportional dependence between body mass and the level of functional limitations and risk of falls.

Conclusion. In our functional tests, we found a statistically significant correlation with regard to age and BMI index.

Keywords. age, BMI, older adults, physical ability, sex

Introduction

The process of ageing is irreversible. An older adult undergoes involutional changes both in the mental and the physical spheres. Gradually, functional ability deteriorates, and difficulties in activities of daily life arise. Numerous needs and interest diminish.[1,2] In spite of changes to the body, the ageing process may be slowed down. In the desired process of ageing there are some necessary elements, and these are: forming healthy habits, avoiding risky behaviour, maintaining the highest possible level of physical activity and maintaining independence. These are key factors in both prevention and treatment.[3,4]

One of the most worrying problems of the 21st century is hypokinesia (the word derives from Greek: kinesis – movement, and hypo – decrease, shortage, limitation). It

Corresponding author: Justyna Drzał-Grabiec, e-mail: justyna.drzal.grabiec@wp.pl

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

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affects developed societies such as that found in Poland. It can be characterized by lack of sufficient physical activity, or the disproportion between the increasing load for the nervous system and the decreasing load for the locomotor system, and it leads to disorders in the functioning of the systems of human body. The World Health Organization declares that hypokinesia is, directly and indirectly, the main cause of death, and that it is constantly increasing.[5]

Physical activity helps to control the pathology of ageing, as it reduces the risk of developing cardiovascular disorders, reduces the development of hypertension, regulates the intestine rhythm thus decreasing fat tissue and helping to avoid obesity, reduces the risk of developing cancer, prevents osteoporosis (bone fractions), increases the muscle strength and muscle endurance, maintains motor and cognitive functions, reduces stress levels and increases self-esteem.

The aim of the study was to assess the level of physical ability and chosen factors that affect physical ability in adults older than 65 years.

Material and method

The study involved 63 older adults. The criterion for inclusion in the study was an age over 65 years. The subjects expressed their written consent to participate in the study. The study population consisted of 37 women (58.7%) and 26 men (41.3%) We used the snowball sampling technique.

The age of the subjects was between 65 and 81 years, the mean age was 73.76 years ± 4.43 years. The mean age for women was 73.18 years, and for men 74.34 years (Table 1).

To conduct this study, we used our own questionnaire. We assessed physical ability using the Short Physical Performance Battery (SPPB). We assessed three areas: lower extremity strength, static balance and gait velocity.[6] To assess functional ability and the risk of falls, we used the Up&Go test.[7] We also calculated Body Mass Index and we checked the subjects for depression.

The subjects were guaranteed anonymity. They expressed their informed consent to participate, and they

Table 1. General characteristics of the study population

Variable	n	%
Professional activity in the past		
Yes	63	100.0
No	0	0.0
Total	63	100.0
The type of professional activity in the past		
Active	12	19.0
Sedentary	23	36.5
Mixed	28	44.4
Total	63	100.0
Lifestyle before turning 65		
Very active (activity at least twice a week)	6	9.5
Moderately active (activity at least once a week)	16	25.4
Minor activity (activity at least twice or three times a month)	23	36.5
Inactive	18	28.6
Total	63	100.0
Frequency of doing physical activity		
Every day	15	23.8
Several times a week	11	17.5
Rarely	22	34.9
None	15	23.8
Total	63	100.0
Chronic diseases in the study population		
Cardiovascular diseases	35	55.6
Pulmonary diseases	14	22.2
Gastrointestinal diseases	13	20.6
Genitourinary diseases	18	28.6
Endocrine system diseases	15	23.8
Locomotor system disorders	36	57.1
Bone fractions after the age of 60 years	3	4.8
Cancer	0	0.0
Other	1	1.6

were presented with the plan of the tests. The order of the conducted tests was the following: filling out the questionnaire, doing and analyzing the SPPB test, doing the Up&Go test and depression assessment.

Table 2. Functional limitations, according to SPPB scale

Functional limitations, according to SPPB scale	n	%
Serious limitations(0-3 points)	2	3.2
Moderate limitations (4-6 points)	10	15.9
Minor limitations (7-9 points)	30	47.6
No limitations (10-12 points)	21	33.3
Total	63	100.0

Table 3. Risk of falls, according to Up&Go test

Risk of falls, according to Up&Go test	n	%
Normal, minimal risk of falls	11	17.5
Average risk of falls	39	61.9
High risk of falls	13	20.6
Total	63	100.0

Statistical analysis

To assess relationships between the chosen variables, questions on nominal scales from the following tests were used: V Cramer test (tables 2×3, 4×5 etc), the Phi test (tables 2×2). These are symmetric measurements, based on the chi-squared tests, and they inform of the strength of relationship between variables in contingency tables. All the measurements for the strengths of relationships have been normalized, so that they take values from the (0-1) range. There are the following strengths of relationships: 0-0.29 denotes a weak relationship, 0.3-0.49 denotes a moderate relationship, 0.5-1 denotes a strong relationship. For numerical values, we calculated descriptive statistics, i.e. the mean, the median, the minimum, the maximum, the first and the third quartiles, and standard deviation. We established statistical significance at $p<0.05$. We conducted the statistical analysis using the software package Statistica 10.0.

Results

There were two subjects (3.2%) in the study population who had serious functional limitations, 10 subjects (15.9%) who had moderate limitations, 30 subjects (47.6%) who had minor limitations and 21 subjects (33.3%) who did not have any functional limitations (Table 2).

There were 11 subjects (17.5%) without risk of falls, or with minimal risk of falls. There were 39 subjects (61.9%) with average risk of falls. There were 13 subjects (20.6%) with high risk of falls (Table 3).

We found a statistically significant relationship between the age of the studied subjects and assessment of their suppleness (p=0.009) and their balance (p=0.043). Both relationships had moderate strength (V Cr=0.32). Younger subjects had better suppleness and lower risk of falls than older subjects (Table 4).

We found a statistically significant relationship between the sex of older adults and their susceptibility to depression (p=0.038). This relationship had moderate strength (V Cr=0.32). Depression was more common in women than in men (Table 5).

We found a statistically significant relationship between the body mass category of the studied older adults and the level of their functional limitations (p=0.034) and their balance (p=0.041). These relationships were: moderate (V Cr=0.33) or weak (V Cr=0.28), respectively. The higher BMI of the studies seniors, the greater their functional limitations. Also, with increasing body mass, the risk of falls of the subjects increased too (Table 6).

Table 4. Functional test results, depending on age

Tests		65-73 years		73-81 years		*P*
		N	%	n	%	
=Depression scale	Normal	17	51.5	20	66.7	$\chi^2(2)=2.77$ p=0.249
	Moderate depression	14	42.4	10	33.3	
	Serious depression	2	6.1	0	0.0	
SPPB	Serious limitations	1	3.0	1	3.3	$\chi^2(3)=0.28$ p=0.962
	Moderate limitations	5	15.2	5	16.7	
	Minor limitations	15	45.5	15	50.0	
	No limitations	12	36.4	9	30.0	
Suppleness	Poor	22	66.7	28	93.3	$\chi^2(1)=6.82$ p=0.009 Phi=0.32
	Good	11	33.3	2	6.7	
Up&Go	Normal. low risk of falls	9	27.3	2	6.7	$\chi^2(2)=6.27$ p=0.043 Phi=0.32
	Average risk of falls	16	48.5	23	76.7	
	High risk of falls	8	24.2	5	16.7	

Table 5. Functional tests results, depending on sex

Tests		Females		Males		*P*
		N	%	N	%	
Depression scale	Normal	17	46.0	20	76.9	$\chi^2(2)=6.52$ p=0.038 V Cr=0.32
	Moderate depression	18	48.7	6	23.1	
	Serious depression	2	5.4	0	0.0	
SPPB	Serious limitations	2	5.4	0	0.0	$\chi^2(3)=1.48$ p=0.685
	Moderate limitations	6	16.2	4	15.4	
	Minor limitations	17	46.0	13	50.0	
	No limitations	12	32.4	9	34.6	
Suppleness	Poor	30	81.1	20	76.9	$\chi^2(1)=0.16$ p=0.688
	Good	7	18.9	6	23.1	
Up&Go	Normal. low risk of falls	6	16.2	5	19.2	$\chi^2(2)=0.75$ p=0.684
	Average risk of falls	22	59.5	17	65.4	
	High risk of falls	9	24.3	4	15.4	

Table 6. Functional tests results, depending on BMI

Tests		Norm		Overweight		Obesity		*P*
		n	%	n	%	n	%	
Depression scale	Normal	8	57.1	18	69.2	11	47.8	$\chi^2(4)=3.04$ p=0.549
	Moderate depression	6	42.9	7	26.9	11	47.8	
	Serious depression	0	0.0	1	3.9	1	4.4	
SPPB	Serious limitations	0	0.0	0	0.0	2	8.7	$\chi^2(6)=13.58$ p=0.034 V Cr=0.33
	Moderate limitations	0	0.0	3	11.5	7	30.4	
	Minor limitations	6	42.9	14	53.9	10	43.5	
	No limitations	8	57.1	9	34.6	4	17.4	
Suppleness	Poor	9	64.3	22	84.6	19	82.6	$\chi^2(2)=2.52$ p=0.282
	Good	5	35.7	4	15.4	4	17.4	
Up&Go	Normal. low risk of falls	4	28.6	6	23.1	1	4.4	$\chi^2(4)=9.98$ p=0.041 V Cr=0.28
	Average risk of falls	9	64.3	17	65.4	13	56.5	
	High risk of falls	1	7.1	3	11.5	9	39.1	

Discussion

Our study confirmed the sedentary lifestyle of older adults. More than a half of the study population performed physical activity rarely, or even declared lack of any physical activity. The results of the study by Maciatowicz were even less optimistic, as he found that 74% of older subjects did not perform any physical activity, and that 12% did it rarely. [8] According to the recommendations of American College of Sports Medicine, to improve the health of an older adult, one has to do physical activity for 30 minutes three times a week.[9]

According to Zadworna-Cieślak et al. the age does not impact a more or less often engagement in physical activity. They only found correlation between sex and health of adult citizens. [10] Zielińska-Więczkowska and Kędziora-Kornatowska found that institutions such as the University of the Third Age prevents the exclusion of older adults, and it significantly increase their activity and self-esteem.[11] Ćwirlej-Sozańska proved that older adults who declared leading an inactive lifestyle have difficulties in performing activities of daily life and in movement, which in turn negatively affect their self-esteem. In her study, Ćwirlej-Sozańska found a significant relationship between physical activity of her study population and difficulties in movement.[12]

All subjects had been professionally active in the past. Usually they had work of mixed character, and least often they had work that required physical activity. According to Zużewicz and Konarska, the process of ageing leads to decreased physical and psychological ability.[13] Cutting down on hard physical work, and on work in difficult conditions, e.g. at night, slows down the ageing process. Performing work in difficult conditions speeds up the ageing process. Decreased ability to perform intellectual work manifests itself later, approximately after 65 years of age, and, in contrast to hard physical and shift work, it is different in different individuals.

Low physical activity in senior citizens may have been caused by their multiple diseases. The most common diseases in the study population were the locomotor system disorders and cardiovascular diseases. More than a half of the subjects had to take prescribed medicines. Approximately 35% of the subjects also used OTC

drugs. Rubenstein and Josephson stressed the multiple diseases in older adults. They believed it was multiple diseases, and not only the involutional process, that is the main reason for reduced muscle strength and decreased physical activity in older adults.[14]

Adamczyk et al believe that it is necessary to educate older adults, as it may result in forming and maintaining high level of physical ability.[15]

Zielińska-Więczkowska and Kędziora-Kornatowska named the level of physical ability as the most important determinant of quality of life of senior citizens. [16] Kocemba et al. believe that the ageing process has an individualized character. The chronologic age does not always appropriately match the biological age of individuals. It is important to engage in preventive activities that delay the ageing process and build physical fitness.[17] A higher body function results in higher self-esteem. Bień reports that a low level of physical fitness in the Polish society is a significant social issue. The reasons for this are lack of preventive actions, the development of automobile industry and computerization.[18]

It worried us that our study found 24 cases of moderate depression and 2 cases of serious depression among our study population. There was a relationship between the sex of the respondents and the incidence of depression - women were more susceptible to it. Bień et al. confirmed it. Depression is one of the most serious diseases of affluence. The constant hurry increases the symptoms. Although the disorder affects all age groups, older adults are particularly sensitive to it.

In the Short Physical Performance Battery (SPPB), which assesses physical ability, the majority of subjects had results that showed minor limitations (30 subjects) or no limitations (21 subjects). The best results were found in the balance trial. As many as 59 subjects scored four points. The gait velocity was on average level - the mean result was 6.7 second, which usually meant a score of two points. The poorest results were found in the chair stand trial. The mean result was 15.1 seconds, which meant a score of 2 points. According to Guralnik et al. the SPPB result helps to determine the risk of disability.[19] Huang et al also used this test to determine the limitations to performing activities of daily living.[20] Miller reported a decreased risk of death for each additional point scored in the test.[21] Protas et al. used SPPB to assess the effectiveness of rehabilitation in older adults.[22]

The Up&Go test results determined that in the majority of subjects (39 persons) the risk of falls was average. The results were therefore in the rage 10-19 seconds. The second biggest group were the subjects with high risk of falls (13 persons) with results higher than 19 seconds. In their study, Adamczyk et al found that the mean time of the test was 5.58 seconds, i.e. normal result, and meant a low risk of falls. In our study only 11 subjects had such results. Osiński et al. mentioned that older people have an increased risk of falls due to reduced muscle strength and deteriorated motor coordination. They believe that approximately half of the falls result in injuries, of which 10-15% are serious injuries to the head, and 5-6% are fractures to the femoral head. Thomby observed that as many as 10-20% of falls in older females lead to death.[24] Cwirlej-Sozańska et al. mention that along with the ongoing tendency of ageing societies, the test of balance in older adults is particularly significant. Moreover, the Up&Go test results correlate with other clinical results - Tandem Walk or Pivot 180º.[25] Nordin et al. found the Up&Go test reliable in assessing the risk of falls, and mention that this test is used as a determinant by the British Geriatrics Society.[26] A review of the literature by Beauchet et al. confirm the theory of the prognostic function of risk assessment among subjects older than 60 years.[27] Our study found a relationship between age and the Up&Go test result. The risk of falls was lower in younger subjects. Beauchet et al. stressed the need to study this correlation, as well as the impact of sex. In our study, the sex did not have impact on results.[27]

Konieczny and Rasińska stated that 40-60% of European Union older adults have sedentary lifestyle.[28] In Poland, physical activity of older adults is very poor. Only 12% of Poles reported they do physical exercise once a week.[29]

The ongoing ageing of societies brings new challenges to the healthcare system. It shall be necessary to take action to activate older adults and to draft a strategy that would result in facilitating the process.

Conclusions

We found a statistically significant correlation in functional tests in relation to sex and to BMI.

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ORIGINAL PAPER

Agnieszka Bejer [1,2] (ABCDFGH), Sabina Mastej [3] (DFG), Elżbieta Domka-Jopek [1] (BCD), Mirosław Probachta [2] (BCD), Marek Kulczyk [2] (CD), Sharon Griffin [4] (AD)

Polish linguistic adaptation of the Western Ontario Shoulder Instability Index

[1] Institute of Physiotherapy, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[2] The Holy Family Specialist Hospital, Rudna Mała, Poland
[3] Department of Rehabilitation, Health Centre Dr Mastej, Jasło, Poland
[4] Fowler Kennedy Sport Medicine Clinic, University of Western Ontario, London, Ontario, Canada

ABSTRACT

Introduction. The Western Ontario Shoulder Instability Index (WOSI) is a patient reported outcome measure to assess the impact of unstable shoulder on various spheres of a patient's life. The aim of this paper is to present the stages of linguistic adaptation of the English version of the WOSI questionnaire into the Polish version (WOSI-PL).

Material and methods. The research used the procedure of translation of research tools recommended by Mapi Research Institute, which allows to minimize errors resulting from the translation.

Results. Two versions of the translation were created: A1V and A2V, and based on them, a common version BV was agreed. An English native speaker made a back translation version – BTV. Next, the BTV was compared with the source version SV, corrections were made and the CV version was created. Based on the analysis of experts' assessments, a DV version was agreed, then evaluated by a group of 6 patients. The patients' responses was analyzed, and the final version – WOSI-PL was created.

Conclusion. The WOSI-PL constitutes a valuable tool to evaluate health-related quality of life in patients with shoulder instability. The questionnaire before being introduced to scientific research and clinical practice will be subjected to a validation process in order to evaluate psychometric properties.

Keywords. questionnaire, quality of life, shoulder instability, WOSI

The list of abbreviations:

A1V – A1 version of Western Ontario Shoulder Instability Index, A2V – A2 version of Western Ontario Shoulder Instability Index, ASES – American Shoulder and Elbow Surgeons Standardized Shoulder Assessment Form, BTV – Back translation version of Western Ontario Shoulder Instability Index, BV – B version of Western Ontario Shoulder Instability Index, DV – D version of Western Ontario Shoulder Instability Index, DASH – Disability of the Arm, Shoulder, and Hand questionnaire, MISS – Melbourne Instability Shoulder Scale, No – Number, OISS – Oxford Instability Shoulder Score,

Corresponding author: Agnieszka Bejer, e-mail: agnbej@wp.pl

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SF-12 – 12-Item Short Form Health Survey, SV – Source version of Western Ontario Shoulder Instability Index, UCLA – University of California Los Angeles *Shoulder Rating scale*, WORC – Western Ontario Rotator Cuff Index, WOSI – Western Ontario Shoulder Instability Index, WOSI-PL – Western Ontario Shoulder Instability Index - Polish version

Introduction

The instability of the shoulder joint is a relatively common complaint, especially among young and physically active patients.[1,2] This term refers to the inability to keep the head of the humerus in the acetabulum of the joint caused by the lack of integrity of both static and dynamic structures that secure the joint. Dislocation may occur as a result of atraumatic instability e.g. as a result of frequently repeated movements causing soft tissue micro-injuries or congenital malformations.[3,4] More often, however, so-called traumatic instability occurs as a result of injuries in contact sports and those in which throw movement are made. Among the various types of post-traumatic instability, anterior dislocation is most frequently observed, which is noted in more than 90% of cases.[5,6] The main symptoms of dislocation are pain, the sensation of popping and clicking in the joint, limitation of physical activity and general decline in quality of life.[7-9]

For a long time, a number of tests have been available differentiating shoulder articular instability and various methods of conservative and surgical treatment. Also measures and tools are increasingly used for subjective assessment of the functional status and quality of life in patients such as Disability of the Arm, Shoulder, and Hand (DASH) questionnaire, Oxford Instability Shoulder Score (OISS), Melbourne Instability Shoulder Scale (MISS) and the Western Ontario Shoulder Instability Index (WOSI).[10-13]

The Western Ontario Shoulder Instability Index (WOSI) was developed by Kirkley A. et al. in 1998. The process of creating WOSI included several stages. Questionnaire questions were created based on a thematic review of the literature, interviews with patients with shoulder instability and health care professionals involved in such patients. Then, the number of items was reduced using patient-generated frequency-importance products and correlation matrices. In the final stage, WOSI was tested by 2 groups of patients of 10 people each. Finally, a questionnaire consisting of 21 questions grouped into four domains was developed. The authors also assessed the psychometric properties of the new tool (validity, reliability and responsiveness). Validation analysis showed that the reliability of WOSI was very high at 2 weeks and 3 months (the intra-class correlation coefficients for the total WOSI score were 0.949 and 0.911, respectively). It was also more responsive (sensitive to change; standardized response mean was 0.931) than five other shoulder measurement tools - the Disabilities of the Arm, Shoulder and Hand scale (DASH); the American Shoulder and Elbow Surgeons Standardized Shoulder Assessment Form (ASES); the University of California Los Angeles *Shoulder Rating scale* (UCLA); the Constant Score; and the Rowe Rating Scale and then a global health instrument – the 12-Item Short Form Health Survey (SF-12) and range of motion.[13] Rouleau DM et al. in a publication that was a systematic review of patient-administered shoulder functional instability showed that out of the 25 questionnaires used in the literature to assess shoulder instability, WOSI appears to exhibit the best psychometric properties for this population.[14]

The selection of an appropriate tool for subjective assessment of the patient in conjunction with an objective assessment of specialists allows to monitor the course of treatment and rehabilitation, and this contributes to the quality of life of the patient.[10] However, for reliable results, standardized research tools must be used.[13,14]

The WOSI questionnaire was translated into German, Dutch, Italian, Japanese, Portuguese used in Brazil, Swedish, Turkish and French.[15-22] We undertook this research due to the lack of research tools in Poland to assess the quality of life of people with shoulder instability or operated for that reason, which would cover different areas of the subjects' lives.

Aim

The aim of this study was to cross-culturally adapt the English version of the WOSI for use in the Polish patients with shoulder instability or operated for shoulder instability.

Material and methods

1. The description of the WOSI questionnaire

The WOSI questionnaire is a specific, self-reported research tool for assessing the quality of life in patients with shoulder instability – in the source version it was developed in English. The questionnaire contains 21 questions divided into four sections. Section A deals with physical symptoms and contains 10 questions, section B contains 4 questions and refers to sport, recreation and work, section C also contains 4 questions and concerns lifestyle and the last section D contains 3 questions and concerns emotions. The WOSI score contains extensive written instruction for users, which includes a clarification of every single question.[13]

2. The assessment of the level of quality of life and functional state of patients with shoulder instability on the basis of WOSI questionnaire

The format of the response to the questionnaire is a 10-cm analogue visual scale on which the respondents put a slash '/'. The score is calculated by measuring the dis-

tance from the left of the scale to the nearest 0.5 mm and converting the result to 100. In this way, the final result of 2100 can be calculated by summing the results from individual sections (Physical symptoms / 1000; Sport / Recreation / Work / 400; Lifestyle / 400; Emotions / 300). The best possible score is 0, which signifies that the patient has no decrease in shoulder-related quality of life. The worst score possible is 2,100. This signifies that the patient has an extreme decrease in shoulder-related quality of life. The score can also be presented as percentage with 100% (0 points) being the best score and 0% (2100 points) being the lowest score. To obtain the final percentage, the total result should be subtracted from 2100 and then divided by 2100 and multiplied by 100. To calculate the percentage for individual sections, the same pattern must be followed.[13]

3. Translation methods and language adaptation applied to the Polish version of WOSI questionnaire

The approval was obtained from the copyright owner – Sharon Griffin – to create a Polish version of the WOSI questionnaire.

The adaptation process of WOSI was taken in accordance with the guidelines of the Mapi Research Institute and including 6 stages:[23]

1. "Forward" translation by two independent translators → forward version AV1 and forward version AV2
2. Reconciliation meeting between the two "forward" translators and the local team → forward version BV
3. "Backward" translation by an independent translator → backward translation BTV
4. Comparison of the source questionnaire with the "backward" translation by the local team, "backward" translator and author → forward version CV
5. Review by clinicians working in the relevant medical field → forward version DV
6. Cognitive debriefing - test of the clarity, understandability and acceptability of forward DV on 6 person with shoulder instability and who are native speakers of the target language → final version – WOSI-PL.

Results

The Polish linguistic adaptation of the WOSI was made in six stages:

Stage I

At this stage of adaptation, translation was used, which is characterized by a high level of translation reliability, but allows the introduction of Polish language equivalents in case of expressions causing problems in translation in the original questionnaire. Two independent versions of the translation - 'forward' version AV1 and AV2 were prepared by two English translators of Polish origin.

Stage II

The team composed of the above-mentioned translators and co-authors of this publication analyzed both individual items, sets of answers to questions, instructions for filling the scale, as well as its graphic layout. Acceptable discrepancies between the two translations were found, and 6 out of 21 questions showed potential problems with their adaptation to the Polish language. They resulted mainly from the lack of equivalent for a given English phrase (question 17) or from a large number of equivalents that a translator could use (question 5,8,9,13,15). Then the team agreed a common version of the translation - 'forward' version BV.

Stage III

The BV version was translated back into English without backing up the text of the original and 'backward' version - BTV was obtained. The procedure was performed by a person whose native language is English, and has been living in Poland for a few years and is fluent in Polish as well.

Stage IV

Co-author of the source version - SV WOSI questionnaire compared the BTV version with the SV version and indicated discrepancies. The comparisons were also made by a translator who prepared the BTV version and co-authors of adaptation. This allowed to verify the BV version in Polish. As a result of detailed analyses, corrections were made and another Polish version of the 'forward' version CV of the questionnaire was created. Table 1 presents the main changes introduced to the Polish version of WOSI. Also, a frequent change was replacing the wording 'Jak znaczne…' ('How significant…') by 'Jak duże…' ('How much…'), as well as a change of tense in a sentence.

Stage V

The team of experts with many years of experience with orthopedic patients reviewed the version of the CV. The team included: 1 specialist in orthopedics, 1 physiatrist and 3 physiotherapists. Experts assessed the conformity of each question in the SV version with the question in the CV version. They compared whether both questions measure the same symptoms or problems that occur during every day, recreational or professional activity. Conformity was assessed on a 6-point scale from 0 to 5, where 0 means that Polish translation is inadequate and 5 is fully adequate. If the expert assessed the question at level 3 or lower, he was obliged to present an alternative proposal (tab. 2). As a result of the corrections made, another Polish version of the 'forward' version of the questionnaire was created.

Table 1. Changes made to the WOSI of the Polish version during stage IV

No	Source version – SV	Backward translated English version - BTV	Back translation review	Polish versions - BV and CV i.e. version obtained as a result of corrections after backward translation
3	How much weakness or lack of strength do you experience in your shoulder?	How significantly can you feel reduction or lack of strength in your shoulder?	How much do you feel a lack of strength in your shoulder?	**[CV]** Jak duże osłabienie odczuwasz w swoim barku? **[BV]** Jak znaczne osłabienie lub brak sił odczuwasz w swoim barku?
5	How much clicking, cracking or snapping do you experience in your shoulder?	How significantly can you feel clicking, friction or popping in your shoulder?	How much clicking or popping do you have in your shoulder?	**[CV]** Jak dużo klikania i strzelania odczuwasz w swoim barku? **[BV]** Jak znaczne klikanie, tarcie i strzelanie odczuwasz w swoim barku?
12	|——————| not affected extremely affected	|——————| lack of influence extreme influence	|——————| not at all extreme	**[CV]** |——————| wcale ekstremalnie **[BV]** |——————| brak wpływu ekstremalny wpływ

Table 2. Conformity assessment of SV of the WOSI with CV on the example of question number 1

No.	Source version SV	Polish version CV	inadequate translation					translation fully adequate	Assessment 0, 1, 2 or 3
1.	How much pain do you experience in your shoulder with overhead activities?	Jak duży ból odczuwasz wykonując czynności wymagających uniesienia rąk ponad głowę?	0	1	2	3	4	5	Write out badly translated word or expression ……...……. Give your suggestions …….

On the basis of the results of the expert assessment, questions 5 and 17 were identified, to which the experts raised objections. They made also small comments to the questions No. 3,4,19,20. The above questions were subjected to an expert discussion, after which the translations were changed. Question No. 5 - the description of the symptoms 'How much clicking and shooting do you feel in your shoulder' was supplemented with the word 'popping'. Question No. 17 the phrase '... when frolicking or fooling around ...' was changed to '... when frolicking or playing ...'. The experts estimated the translation of the remaining questions as fully adequate.

Stage VI

At the last stage, the DV version of the WOSI questionnaire was tested on a group of six patients - 3 women and 3 men between the ages of 20 and 33 (average 27.2 years). Shoulder instability occurred in 3 subjects, while 3 people were subjected to surgery due to shoulder instability. Ailments have been present for at least 3 months (on average 4.6 months, 3-6 months). Therefore, they had appropriate experience in functioning in everyday life with an unstable shoulder or a operated shoulder, so as to reliably assess the accuracy of questions and the clarity of their wording. The task of the testers was to complete the questionnaire and indicate whether the given questionnaire item and the scale used to answer the questions was fully understood or raised doubts. Answers were rated on a four-level scale, where: 3 means that the question is perfectly understandable, 2 - the question is completely understandable after reading the explanations to the questions, 1 - the question is only partly understandable, even after reading the explanations to the questions, and 0 - the question completely incomprehensible. At the same time, when the question was incomprehensible to the respondent, they were asked to indicate the reason for the lack of understanding.

Analyzing the obtained answers from 6 people, the average score was 2.94. The average grade indicated by the respondents in question 7, 9 and 19 is 2.56 points. The remaining items obtained the maximum score from all testers (3.0).

Questions that raised doubts are: 7. How much discomfort do you feel in your neck muscles due to shoul-

der problems? 9. How much do you use other muscles to compensate for your shoulder problems? 19. How aware are you of the problems regarding your shoulder?

The respondents stated in the survey that the above-mentioned questions are completely understandable after reading the explanations to the questions placed at the end of the questionnaire. Therefore, the experts decided that the information which is in the instructions for patients section: 'If for some reason you do not understand the question, please refer to the explanations at the end of the questionnaire', it will be bolded so that the patient will keep it in mind.

The respondents also assessed the readability of the instructions for completing the WOSI questionnaire, the degree of understanding the explanations to questions, the transparency of the graphic layout, and the complexity of presenting problems in the questionnaire in various areas of life related to the unstable shoulder. They provided answers to the above questions in a scale from 0 to 3, where 3 means full understanding of the questionnaire instructions and explanations to the questions, approval of the layout and full exhaustion of the topic, and 0 is ambiguity of instructions and the lack of explanations to questions, inadequate graphics and covering the topic cursory. According to all the surveyed, the instructions for completing the questionnaire and explanations of the questions are fully understood (average 3 pts), and the questionnaire addresses all areas of life affected by problems related to the unstable shoulder for 5 people and partly to 1 person (2 pts). They also determined the time needed to read the instructions and complete the questionnaire, which averaged 6.5 minutes, in the range of 5 to 9.5 minutes.

After the team of experts had analyzed the patients' responses, corrections were made and the questionnaire assumed the shape of its final version – WOSI-PL (see appendix 1).

Practical considerations

The mean time required to calculate the result obtained on the basis of the WOSI questionnaire is 228 seconds, within the range from 182 to 277 seconds. It was given on the basis of 3-fold calculations for 6 questionnaires completed separately by 5 physiotherapists.

Discussion

Cross-cultural adaptation and validation of measurement tools enables a standardized comparison of test results, thus helping to optimize treatment strategies.

In the case of shoulder instability, the WOSI questionnaire is a measure which is reliable, accurate and sensitive to changes in the patient's condition and officially approved in nine languages to assess patients with shoulder instability.[13,15-22]

Cultural adaptation and translation of WOSI to the Polish version took place according to the guidelines of the Mapi Research Institute, which were in accordance with the procedures suggested by the authors of the source version. Similarly to the Western Ontario Rotator Cuff Index (WORC), adapted to the Polish version by Bejer A. et al. 2017, WOSI conducted a double translation process into Polish and a reverse translation.[23,24] During the creation of the forward version BV, potential problems with adaptation to the Polish language were shown in 6 out of 21 questions, which resulted mainly from too many equivalents for a given English phrase, or lack of such equivalent in question No. 17. Barbosa G et al. had a similar problem in question No 17 by creating the Brazilian version of WOSI.[19] Eventually, the disputable issues were resolved during the creation of the final version based on expert discussions and testing the questionnaire in the final stage of development on a group of 6 patients diagnosed with shoulder instability.

The multistage of conducted research in accordance with international guidelines and the full involvement of the author of the source version resulted in the creation of a well-translated and complete version of the Polish WOSI. It also seems reasonable to try to examine the psychometric characteristics of the questionnaire, which are of key importance in its application.

The Polish version of the WOSI questionnaire is available free of charge for scientific research exclusively with the consent of the WOSI co-author – Sharon Griffin. Please send correspondence to the following e-mail address: sgriffinlaity@gmail.com. This version of the WOSI-PL is attached to this publication. It can be also downloaded from the website of the Holy Family Specialist Hospital in Rudna Mała: http://www.klinika-rzeszow.pl.

Conclusion

1. The Polish version of the Western Ontario Shoulder Instability Index was accepted by the copyright owner and constitutes a valuable tool to evaluate health-related quality of life in patients with shoulder instability.
2. The Polish language version of the Western Ontario Shoulder Instability Index before being introduced to scientific research and clinical practice will be subjected to a validation process in order to assess psychometric properties.

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REVIEW PAPER

Marzena Skrzypa [1(ABDFG)], Natalia Potocka [1(ABDFG)], Halina Bartosik-Psujek [2,3(ADFG)], Izabela Zawlik [1,2 (ABDFGH)]

Genetic risk factors of Alzheimer's disease

[1] Laboratory of Molecular Biology, Centre for Innovative Research in Medical and Natural Sciences, Faculty of Medicine, University of Rzeszow, Rzeszow, Poland
[2] Institution of Experimental and Clinical Medicine, Faculty of Medicine, University of Rzeszow, Rzeszow, Poland
[3] Clinical Department of Neurology Rzeszow State Hospital No. 2, Rzeszow, Poland

ABSTRACT

Introduction. Alzheimer's disease (AD) is one of the most common neurodegenerative diseases, which is a serious health problem for societies that live longer. Spontaneous dominant mutations and polymorphisms of selected genes play an important role in development of AD.

Aim. Several polymorphisms in selected genes strongly associated with development of Alzheimer's disease were highlighted in this review: *APOE, CYP46, APP, PSEN1, PSEN2, UBQLN1, BACE1, PRND, APBB2, TOMM 40*. These gene polymorphisms have a significant role in the development of Alzheimer's disease and they have potential to be biomarkers. Researchers combine efforts to find significant polymorphisms that would ensure that a person is predisposed to the occurrence of disease symptoms. This topic is often taken up by scientists seeking to develop effective genetic tests for diagnosing AD.

Material and methods. Analysis of literature from web of knowledge: Web of Science (all database), NCBI and PubMed.

Results. We reviewed the selected important genes and polymorphisms which are most often associated with development of AD.

Conclusion. It should be noted that nowadays scientists strive not to focus on only one polymorphism in the gene but on several polymorphisms in different genes concomitantly and above all on interactions between them to the diagnosis of this disease. Only this approach to AD will contribute to the creation of appropriate identification methods. Moreover, we should use the new generation tools - the platform for collecting data and personalized medicine.

Keywords. autosomal genetic mutations, early-onset Alzheimer Disease, genetic polymorphisms, late-onset Alzheimer Disease

Introduction

Alzheimer's disease general characteristics

Alzheimer's disease is the most common form of dementia. It affects 24.3 million people around the world. To date, over twenty genetic loci have been associated with AD and a significant number of genetic variants were mapped within these loci. A large part of important genetic variants lies outside the coding region (in introns). However, the reliable function of these variants is still under unexplored.[1-3]

Corresponding author: Izabela Zawlik, e-mail: izazawlik@yahoo.com

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

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Alzheimer's disease is one of many neurodegenerative diseases that appear in a population of societies that live longer. In spite of many years of intense search, no unequivocal genetic and biochemical markers have been found that would allow for the in vivo differentiation of AD among many other dementia syndromes. Due to the variety of symptoms and the course of AD, and along with the development of molecular techniques, a different approach to the disease began to be looked at: as a polygenic disease (polymorphisms), methylation of histone proteins of selected genes, and splicing methods.

In Verheijen's review, particular attention was paid to GWAS (genome-wide association studies), TWAS (transcriptome-wide association studies) and EWAS (epigenome-wide association studies).[4]

The review of scientific articles provides us with a wider spectrum of opinions on this health problem and sees them as an opportunity to faster diagnose and prevent the development of AD.

Several forms of Alzheimer's disease are distinguished due to family conditions and the age of the patient during the first symptoms of the disease. Classification based on the number of people affected by dementia in the patient's family. We can distinguish the family form Alzheimer disease (at least 2 people in the patient's family – FAD) and the sporadic form Alzheimer disease (without family conditions – SAD). It is estimated that in 15-40% of cases the disease may have family conditions. Only a few percent of all FAD cases show a clear autosomal dominant AD inheritance pattern. The sporadic form predominates in the patient population (60-85%) with a more complex type of inheritance and a multifactorial etiology.

Depending on whether the first symptoms occurred in the patient before 65 years of age or after 65 years of age there is an early form (early onset Alzheimer disease: EOAD; ≤65 years) and late form (late onset Alzheimer disease: LOAD; >65 years). EOAD is revealed in 2% of cases, characterized by impaired dexterity, impaired ability to interpret sensory impressions, memory disorders, associative problems, personality changes and degeneration of the frontal and parietal lobes.[5] In 98% of people with LOAD, memory disorders prevail, while the dexterity and the ability to interpret sensory impressions are less damaged.[6] In LOAD, clinical symptoms result from extensive cortical atrophy. The duration of the disease is usually 5-12 years. Death occurs as a result of non-cerebral complications (pneumonia, decubitus ulcers). Research focuses not only on the family form of the disease but also on spontaneous, dominant mutations, which were formerly ignored in scientific research. It is noted that they can lead to the first symptoms of the disease, even before the age of 30 named young onset Alzheimer disease (YOAD).[7,8]

There are several reasons for the occurrence of AD disease: genetic determinants, lifestyle, diet, interpersonal contacts. It was found that the more we lead an active lifestyle (we undertake to solve problems, we educate ourselves, we run a social life, we have a family, we develop our interests, for example: we solve crosswords, read books) the later we can observe the development of the disease. In Alzheimer's disease we distinguish various stages of disease: mild cognitive impairment (MCI), and: AD I, AD II, AD III.[9] Clinical diagnosis of MCI and different stages of AD is based on laboratory tests and medical history combined with the exclusion of symptoms typical of other dementias (vascular dementia (VaD), frontotemporal dementia (FTD), pseudo dementia).[10] Changes in Alzheimer's disease relate to cognitive function disorders, memory, disorders in the field orientation, coping with stress and problems with everyday life. One of the symptoms of the disease is isolation from the environment. Morphologically the pathological changes of brain are observed: in the brain size (losses in the amount of tissue 1-2% per year), changes in the hippocampus and sometimes in the thickness of the gray matter.[11]

Usually diagnostic research uses cerebrospinal fluid (CSF) to determine typical markers in AD. Morphologically in the diseased tissue are observed: amyloid plaques, intraneuronal deposits of spirally Tau protein fibers, granular-filamentous degeneration (hyperphosphorylated Tau protein) and hirano bodies (e.g. cytoplasmic, eosinophilic, rod-shaped structures found inside nerve cells witch are built from actin and proteins). Rarely are observed: neuron atrophy, synapse degeneration, brain atrophy (cerebral tissue rupture). Changes confirming the diagnosed form of Alzheimer's disease are often observed *post mortem* - after the patient's death.[2]

Genetic polymorphism

The genetic determinants play an important role in the development of AD disease. Over 20 genes responsible for the development of this disease have been detected. Knowledge of genetic predisposition may affect a person at risk of developing Alzheimer's disease to take appropriate steps in a lifestyle change and early treatment to delay the development of the disease. Genetic polymorphisms that cause changes leading to dementia concern codons of genes (exons) and non-coding sequences (introns). Modern research also focuses on mitochondrial inheritance. In genetics, polymorphism means the occurrence of differences DNA in the population. However, polymorphism is not defined as rare phenomenon (1% or higher frequency in the population).[12]

Polymorphism can be divided into:

– SNP (Single Nucleotide Polymorphism) - a single nucleotide polymorphism consisting in exchanging

one base for another in the DNA strand. The phenomenon of single nucleotide polymorphisms is identified by the RFLP PCR (Restriction Fragments Length Polymorphism PCR) or HRM PCR (High Resolution Melting PCR) technique;
- polymorphism of tandem repeats – variable number of tandem repeats (VNTR) (e.g. in the mitochondrial gene (mtDNA) – e.g. gene *Tomm 40*);
- insertion/deletion (I/D) polymorphism of larger fragment nucleotides (e.g. in the *ACE, VEGF* genes);
- polymorphism of mini- and microsatellite sequences (short tandem repeats – STR) – it is estimated that there are approximately several thousand loci containing human-genome sequences in the human genome, accumulated mainly in telomere chromosomal regions, containing from 7–100 base pairs in one repetition and about 2 – to several hundred sequentially ordered repeats in one loci. The level of mutations in mini satellite sequences is very high and amounts to about 5%. Microsatellite sequences are a different type of polymorphism, consisting in the presence of a variable number (5–100) of tandem repeats composed of 1 to 6 nucleotides (e.g. (CATG) n or (CA) n), distributed evenly every 6-10,000 base pairs. Different STR alleles usually occur in a population of several to a dozen times, which significantly increases the resolving power of these methods compared to the SNP variability analysis. In the human genome, they are most often located in sequences flanking genes and introns (noncoding areas).

Gene polymorphism means the occurrence of various variants of a given gene, which in consequence may lead to differences in the structure and action of the protein encoded by this gene (non-synonymous mutation - it involves changing the amino acid in the protein). In the case of a silent mutation - synonymous, there is no change in the amino acid in the protein, this is due to the fact that the genetic code is degenerate. On the other hand, changes in non-coding sequences (in introns) may lead to changes in protein expression or may not affect their expression and structure.[13]

Genes that play a major role in AD

Polymorphism of the *APOE* gene (apolipoprotein E) is the first risk factor identified in AD discovered in 1993.[14] In the brain, it plays a role in the metabolism of lipoproteins and cholesterol homeostasis. The gene is located on chromosome 19ql3.2 and occurs in the form of multiple alleles ε2, ε3, ε4 encoding individual isoforms, ApoE2, ApoE3 and ApoE4, respectively. The presence of three *APOE* alleles determines the occurrence of six genotypes in the human population, including three homozygous (2/2, 3/3, 4/4) and three heterozygous (2/3, 2/4, 3/4). The human population is dominated by a 3/3 homozygous genotype (approximately 60%), while Apo 3/4 heterozygotes account for approximately 20% and Apo 3/2 approximately 13%. The remaining genotypes homozygous and heterozygous are a minority, Apo 4/4 2-3%, Apo 2/2 1%, Apo 2/4 1-2%.[15]

The best known variant of the apolipoprotein E (*APOE4*) gene is responsible for the development of the AD. It is a gene coding for a molecule (apolipoprotein) that carries cholesterol in the blood.
- ApoE2 – reduces the chances of getting sick. It occurs in about 10% of healthy people and in 2% with Alzheimer's disease. If a person with ApoE2 becomes ill, it will happen later than in a person without this variant,
- ApoE3 – the most common variant, most likely without affecting the risk of disease,
- ApoE4 – increases the likelihood of developing Alzheimer's disease. It is present in 40% of patients with late form and in about 25% of healthy people. There are genetic tests to detect this variant, but their use is very controversial.[16]

The individual isoforms of ApoE are not evenly distributed among the world's population. Isoforms ε4 and ε2 are more common in Africa and relatively rare in Asia.[17] In Europe, ε4 isoform is more common in the northern countries, unlike in the Mediterranean region, where it is rare.[18] The main place of ApoE synthesis are cells liver, where most of the apolipoprotein E present in the plasma is formed (66-75%). Smaller amounts of ApoE are synthesized outside the liver, in many different peripheral cells, mainly macrophages, astrocytes, but also in the lungs, kidneys, spleen, and muscles. The concentration of apolipoprotein E in healthy people's plasma ranges from 0.016-0.17 g/l.

ApoE compound with pathological disorders

Levels of ApoE are usually measured in plasma and CSF. Peripheral blood ApoE levels have been proposed as biomarkers of AD, but tend to be lower in patients with AD than in healthy individuals. Such findings remain controversial. For this reason Wang *et al.* in their meta-analysis re-examined the potential role of peripheral ApoE in AD diagnosis. Wang *et al.* supports a lowered level of blood ApoE in AD patients. They reportered that value of ApoE as an important risk factor for AD.[19]

The main role plays presence of the ε4 allel, recognized such a risk factor for Alzheimer's disease.[15,20,21] The presence of at least one ε4 allele was found in 80% of familial and 64% of sporadic Alzheimer's disease, while in healthy people, ε4 is approximately 31%. It is estimated that in people homozygous ε4/ε4 symptoms of the disease appear on average 16 years earlier than in non-carriers of this allele.[22] The increased frequency of the ε4 allele was also found in patients with other neurodegenerative diseases, including AD with Lewy bodies, mixed dementia

and mild cognitive impairment.[23-25] There was a consistent association between the presence of an ε4 allele and both the clinical diagnosis of dementia and cognitive decline. These findings confirm a genetic heterogeneity in late onset sporadic AD and prompt caution in the use of ApoE genotype to predict an elderly individual's susceptibility to either dementia or cognitive decline. In addition to the *APOE* gene and its product - apolipoprotein, the Tau protein and the phosphorylated tau protein also play an important role in AD.[26]

Another important genes in pathology AD

Identification of specific risk genes in AD is problematic because the increase risk conferred by a single gene is small. In addition, there is need to identify combinations of demention risk alleles not just individual genes. The complicating factor is also the heterogeneity of the underlying pathological changes, particularly concurrent cerebrovascular disease.[27] Multiple genetic and environmental factors regulate the susceptibility to AD. There is also a common belief that, apart from the best characterized risk factor, the APOE polymorphism, some other genes are involved in sporadic AD susceptibility for example *CYP46, APP, PSEN1, PSEN2, UBQLN1, BACE1, PRND, APBB2, TOMM 40*. To date, numerous genes have been tested for their putative influence on AD; the investigations have been focused mainly on polymorphisms located in particular genes of interest and in intron sequences. Current data on case-control genetic studies are being collected and updated in the AlzGene Database.[28,29]

A gene encoding a cholesterol degrading enzyme of the brain, called 24-hydroxylase (CYP46A1) is located on chromosome 14q32.1 and it has been linked with risk for AD. The single nucleotide polymorphism (T/C) there is in intron 2 of *CYP46* gene. The product of the CYP46 gene is the water soluble 24(S)-hydroxycholesterol. The elevated plasma and CSF hydroxycholesterol concentrations have been found in AD. This is associated with increased cholesterol turnover in brain or neurodegenerative process. Results of analyses of the connection between *CYP46* polymorphisms and AD carried out so far have been incredibly divergent. Both CC and TT genotypes of the rs754203 polymorphic site were proposed as genetic risk factors for AD; whereas other analyses showed no association between this polymorphism and AD.[30-32]

In the research of Golanska *et al.,* Juhasz *et al.* and Combarros *at al.* the authors pointed to the *CYP 46* gene (cholesterol 24S-hydroxylase) as a potential marker for the identification of AD. The *CYP* 46 gene is responsible for the removal of excess brain cholesterol by hydroxylation. The effect of *CYP46* gene polymorphisms on AD has been mainly focused on the known intronic single nucleotide polymorphism (SNP) rs754203.

In the study Golanska *at al.* were found a relationship between polymorphism *CYP 46* rs754203 (CC) and APOE ε4.[30] They analyzed polymorphisms in 215 Polish AD cases and 173 healthy individuals. It was noticed that the CC genotype of the known rs754203 polymorphic site might be a risk factor for AD, especially in at least one APOE ε4 carriers.

These observations were in contrast to results of other authors, who did not find any significant difference in *CYP46* genotypes frequencies between AD and controls after stratification by *APOE* status, although they found an association between the *CYP46* CC genotype and higher risk of AD.

Other conclusions are presented by Anna Juhasz *et al.* in their research. A case–control study was performed on 125 AD and 102 age- and gender-matched control subjects from Hungary, to test the association of *CYP46* T/C and apolipoprotein E (ApoE) gene polymorphisms in AD. They indicate that the intron 2 T/C polymorphism of *CYP46* gene (neither alone, nor together with the *ε4* allele) does not increase the susceptibility to late-onset sporadic AD in the Hungarian population.[31]

In another studies Golanska *et al.* (2009) were found that the levels of phosphorylated tau protein and p-amyloid peptide in CSF were increased in AD patients carrying the rs754203 TT genotype. Analysis of polymorphism distribution carried out in various ways up to now, however, does not reveal a clear relationship between *CYP46* and AD genotypes.[29]

A case-control study Combarros's *et al.* utilizing a group of 321 sporadic AD patients and 315 control subjects was performed to examine the relationship between different genotypes in gene *CYP* 46.Their results indicated too that the intron 2 *CYP46* CC genotype may predispose to AD, and this association is independent of the apolipoprotein E genotype.[32]

For familial form of early-onset Alzheimer's disease, mutations in 3 genes have been described: amyloid precursor protein (*APP*), presenilin proteins (*PSEN1* and *PSEN2*) but they account only a small fraction of all AD cases. Mutations in these three genes maybe cause autosomal dominant forms of EOAD.[33] Although these genes were identified in the 1990s, variant classification remains a challenge, highlighting the need to colligate mutations from large series. Mutations in the gene for *APP* account for about 10-15% of all cases of familial, autosomal dominant form of Alzheimer's, in *PSEN1* - 20-70%, and mutations in *PSEN2* have been described so far only in single families and are considered very rare.

PSEN1 a gene was found on chromosome 14 (gene ID: 5663 NCBI), gene *PSEN2* on chromosome 1 (gene ID:5664 NCBI) and gene *APP* (amyloid beta precursor protein) on chromosome 21(gene ID: 351 NCBI). Patients with Alzheimer's disease (AD) with an inherited

form of the disease carry mutations in PSEN1, PSEN2 or the APP. These disease-related mutations cause increased production of the longer form of amyloid beta (Aβ) the main component of amyloid deposits found in AD brains). Presenilins are thought to regulate the processing of APP through their effects on gamma-secretase, an enzyme that cleaves APP.

APP amyloid beta precursor protein gene encodes a cell surface receptor and transmembrane precursor protein that is cleaved by secretases to form a number of peptides. Some of these peptides are the protein basis of the amyloid plaques found in the brains of patients with Alzheimer disease. Mutations in this gene have been implicated in autosomal dominant Alzheimer disease and cerebroarterial amyloidosis (cerebral amyloid angiopathy). Multiple transcript variants encoding several different isoforms have been found for this gene.

It is also believed that presenilins participate in the cleavage of the Notch receptor, such that they either directly regulate gamma secretase activity or act as protease enzymes themselves. Two alternatively spliced transcript variants encoding different isoforms of *PSEN2* have been identified. The mentioned genes are therefore further candidates as markers of Alzheimer's disease.[34] In the Lanoiselée *et al*. work we read that *PSEN1*, *PSEN2* and *APP* mutations are autosomal dominant forms of EOAD. Although these genes were identified in the 1990s, but need to colligate mutations from large series of familial and sporadic case. Their findings suggest that a unnoticeable part of *PSEN1* mutations occurs *de novo*, which is very importance for genetic counseling, as *PSEN1* mutational screening in autosomal dominant AD because this test is currently performed in familial cases only.[35] The *PSEN1* c.236C>T, p.(Ala79Val) substitution is currently considered pathogenic and to be associated with a LOAD compared to the other *PSEN1* variants. This substitution was also found in subjects with EOAD so its frequency is of importance for diagnosis in autosomal dominant form of AD. They identified only one novel *PSEN2* mutation, c.850A>G, p.(Arg284Gly), and a previously known mutation, p.(Thr122Pro), during this screen. In the *APP* gene, no novel mutation was found too. Researchers also identified a previously reported mutation from autosomal dominant EOAD families. The most frequent one was the c.2149G>A, p.(Val717Ile) substitution and the c.2137G>A, p.(Ala713Thr) mutation was found in other patients from unrelated families. In addition, Lanoiselée *et al*. found families carried mutations located within the coding sequence of the Aβ peptide: "Flemish" *APP* mutation c.2075C>G, p.(Ala692Gly), the "Italian" mutation c.2077G>A, p.(Glu693Lys), and the "Iowa" mutation c.2080G>A, p.(Asp694Asn). In the case of *APP* gene duplication, it was noticed that all patients exhibited progressive cognitive impairment in autosomal dominant EOAD families and in the sporadic cases.[35] Similar results the effects of mutations of the *PSEN1* gene were shown in another research. Bagyinszky *et al.* reports that *PSEN1* mutation (c.335C>T), p.(Thr116Ile) was observed in two Korean families with autosomal dominant inheritance. The personality changes occurred in their 30 years old. This mutation (c.335C>T), p.(Thr116Ile) was first discovered in an Italian patient and two French families with EOAD with similar age of onset. The possible pathogenic mechanisms of mutation were verified. These were changes in the presenilin protein. In addition pathogenic mutation, *PSEN1* (Thr116Asn), was also found where the patient presented young onset AD (YOAD). Its mean that first symptoms appeared before the age of 30.[8]

Similar results were presented in study Giau *et al.* They carried out research on a 37-year-old man, a patient from Korea carrying the mutation *PSEN*1 (p.Gly-417Ala) mutation with exceptionally early and severe presentations, including a wide range of atypical symptoms of rapid cognitive decline with a stooped posture, rigidity, and bradykinesia.

A targeted next-generation sequencing was performed on the patient which revealed a new nucleotide substitution (c1250G> C) in exon 12 of the *PSEN1* gene, changing glycine to alanine at position 417 (p.Gly-417Ala). This mutation may cause disturbances in the 8th transmembrane region, disrupting its functions from the increased hydrophobicity and amount of alanine with reduced elasticity. Since several glycine> alanine substitutions in other transmissive *PSEN1* helices have revealed aggressive phenotypes of Alzheimer's disease, *PSEN1* Gly417Ala may have a common pathogenic mechanism.[36]

Haapasalo *et al.* also reported that key events in the pathogenesis of different neurodegenerative diseases are abnormal protein aggregation and intracellular or extracellular accumulation of misfolded and deposited proteins. Additionally, stress in endoplasmic reticulum and debility of the ubiquitin–proteasome system probably contribute to neurodegeneration in these diseases also in AD. Evidence shows that the AD-associated presenilin also creates aggregates under certain conditions and that ubiquilin-1, controls protein aggregation and their deposition.[37]

The *UBQLN1* gene was found on chromosome 9 and encodes an ubiquitin-like protein (ubiquilin) containing an N-terminal ubiquitin-like domain and a C-terminal ubiquitin-associated domain. The studies suggested that a single intronic C/T polymorphism, UBQ-8i (rs12344615), contribute to AD risk. An association was found between a functional ubiquitination machine and a proteasome to affect protein degradation *in vivo*. This ubiquilin has also been shown to modulate

accumulation of presenilin proteins, and it was found in lesions associated with AD. Two transcript variants encoding different isoforms have been found for this gene. (gene ID: 29979 NCBI)

Single nucleotide polymorphisms in the ubiquilin-1 gene may confer risk for LOAD. El Ayadi *et al.* have shown previously that ubiquilin-1 functions as a molecular chaperone for the amyloid precursor protein (APP) and that protein levels of ubiquilin-1 are decreased in the brains of AD patients. They have recently found that ubiquilin-1 regulates APP trafficking and subsequent secretase processing by stimulating non-degradative ubiquitination of a single lysine residue in the cytosolic domain of APP. Thus, ubiquilin-1 plays a central role in regulating APP biosynthesis, trafficking and ultimately toxicity. As ubiquilin-1 and other ubiquilin family members have now been implicated in the pathogenesis of numerous neurodegenerative diseases, these findings provide mechanistic insights into the central role of ubiquilin proteins in maintaining neuronal proteostasis.[38] Results from Zhang *et al.* from a meta-analysis suggest that the *UBQ-8i* polymorphism may be associated with AD development. But further studies with larger group of respondents are needed to further estimate the presence of an association.[39] The same results were shown by Chuo *et al.* in their research.[40]

An important role in AD also played gene *BACE1*. Aβ is generated from amyloid precursor protein (APP) by β-site APP-cleaving enzyme 1 (BACE1) and γ-secretase-mediated cleavages. Ubiquilin-1, a ubiquitin-like protein, genetically associates with AD and affects APP trafficking, processing and degradation. Natunen *et al.* have investigated ubiquilin-1 expression in human brain in relation to AD-related neurofibrillary pathology and the effects of ubiquilin-1 overexpression on *BACE1*, tau, neuroinflammation, and neuronal viability in vitro in co-cultures of mouse. Taken together, these results suggest that ubiquilin-1 may mechanistically participate in AD molecular pathogenesis by affecting BACE1 and thereby APP processing and Aβ accumulation.[41]

It should be noted that for several years scientists have been some similarities between the background of neurodegenerative diseases such as AD or Parkinson's disease (PD) and prion diseases due to the fact that in both cases protein conformation and aggregation change are observed.[42] *PRND* gene was found on chromosome 20, mutations in this gene may lead to neurological disorders (gene ID: 23627 NCBI). The protein encoded by this gene is a membrane glycosylphosphatidylinositol-anchored glycoprotein. It there is approximately 20 kbp downstream of the gene encoding cellular prion protein, to which it is biochemically and structurally similar.[43]

APBB2 an amyloid beta precursor protein binding family B was found on chromosome 4. The protein interacts with the cytoplasmic domains of amyloid beta precursor protein (APP) and amyloid beta (Aβ) precursor-like protein 2 (APLP2). This protein which is thought to function in signal transduction, contains two phosphotyrosine binding (PTB) domains. Polymorphisms in this gene have been associated with AD.[44] Alternative splicing results in multiple transcript variants. (gene ID: 323 NCBI)

TOMM 40 also known as *PEREC1*-mitochondrial gene is responsible for translocase of outer mitochondrial membrane 40 is located on 19 chromosome (NCBI gene ID: 10452). Mitochondria are semiautonomous organelles. They are responsible for energy production (adenosine triphosphate - ATP). In addition mitochondria play a crucial role in mediating amino acid biosynthesis, in metabolism of lipid, intermediate metabolic pathways, calcium homeostasis and free radical scavenging. These organelles are semi-conservative, and their divisions do not depend on cell division. Their metabolism is important for cell survival and proper functioning. Mitochondrial dysfunction leads, among other things, to neurodegenerative diseases, including Alzheimer's disease. In AD, we first observe mitochondrial dysfunction and then the detectable amyloid pathology.[45,46] *TOMM 40* is the gene which plays an increasingly important role in the pathogenesis of AD. The protein encoded by this gene is localized in the outer membrane of the mitochondria. It is the channel-forming subunit of the translocase of the mitochondrial outer membrane (TOM) complex that is essential for import of protein precursors into mitochondria. Alternatively spliced transcript variants have been found for this gene. In the *TOMM40* gene, the rs10524523 ("523") variable length poly-T repeat polymorphism has more recently been associated with similar phenotypes AD.[47] A variable-length poly-T variant in intron 6 of the *TOMM40* gene, is associated with risk and age of onset of sporadic LOAD. In Caucasians, are distinguish the three predominant alleles at locus in intron 6: Short (S), Long (L) or Very long (V) On an APOE ε3/3 background, the S/VL and VL/VL genotypes are more protective than S/S.[45] Research have shown that impaired mitochondrial function leads to reduced glucose uptake in older individuals, and can lead to insulin resistance, thus perpetuating the cycle linking *TOMM40*, diabetes mellitus (DM), and dementia. Compared to the *TOMM40* short (S) allele, the very long (VL) allele is associated with earlier onset of Alzheimer's dementia, smaller brain volumes, and poorer cognitive performance. Based on the research results we can say mitochondria plays the role in inherited neurodegenerative diseases and they are inherited from the maternal line.[46,48]

In table 1 are included genes polymorphisms very important in increased risk of developing Alzheimer disease.

Table 1. Genome significant representative SNPs reported for AD
NA* – Not available

No.	Genes	ID	located on chromosome	protein coding	single nucleotide polymorphisms (rs) ID	description of the mutation	Role in pathomechanism
	APOE	348	chromosome 19ql3.2	Apolipoprotein E	rs429358 rs7412	three alleles APOE: ε2, ε3, ε4	amyloid beta plaques, which accumulate in the brains of human; familial and sporadic Alzheimer's disease ApoE4 increases the likelihood of developing AD
	CYP46A1	10858	chromosome 14q32.1	a member of the cytochrome P450 superfamily of enzymes	rs754203 rs4900442 (only in the Chinese population)	T/C	Increased cholesterol turnover in brain or neurodegenerative process
	APP	352	chromosome 21q21.3	amyloid precursor protein	NA* NA* NA* NA* NA* rs2830077	G/A p.(Val717Ile) G/A p.(Ala713Thr) G/A p.(Glu693Lys) G/A p.(Asp694Asn) C/G p.(Ala692Gly) C/A	longer form of amyloid beta (Aβ); inherited form of the disease: autosomal dominant EOAD/YOAD
	PSEN1	5663	chromosome 14q24.2	presenilin proteins are component of gamma secretase	rs63750730 rs63750231 rs63750306 rs63750083 rs63751037 rs63750577 NA*	C/T A/C A/C C/A A/G C/T G/C (p.Gly417Ala)	longer form of amyloid beta (Aβ); inherited form of the disease: autosomal dominant EOAD/YOAD
	PSEN2	5664	chromosome 1q42.13	presenilin proteins are component of gamma secretase	rs28936379 rs28936380 rs367855127 rs533813519 rs574125890 rs63750215 rs63750666 NA*	A/G C/G C/T A/C G/T A/T C/T A/G p.(Arg284Gly)	longer form of amyloid beta (Aβ); inherited form of the disease: autosomal dominant EOAD/YOAD
	UBQLN1	29979	chromosome 9q21.32; 9q21.2-q21.3	ubiquitin-like protein (ubiquilin)	rs12344615	C/T	a central role in regulating APP biosynthesis, ubiquilin has been shown to modulate accumulation of presenilin proteins; risk for LOAD
	BACE1	23621	chromosome 11q23.3	APP beta-secretase	rs4938369 rs638405	T/C C/G	the first step in the formation of amyloid beta peptide from amyloid precursor protein
	PRND	23627	chromosome 20p13	membrane glycoprotein	NA*	C/T 3'UTR (untranslated region)	behavioral abnormalities (an elevated risk for delusions, anxiety, agitation/aggression, apathy and irritability/emotional ability)
	APBB2	323	chromosome 4p14-p13	amyloid beta precursor protein binding family B	rs13133980	C/G	LOAD disease onset before 75 years of age characterized pathologically by neurofibrillary tangles and amyloid plaques and clinically by progressive impairment of mental functioning
	TOMM 40	10452	chromosome 19q13.32	Mitochondrial gene; translocase of outer mitochondrial membrane 40	rs10524523	poliT	EOAD and sporadic familiar LOAD

In addition to the polymorphisms of selected genes, histone proteins, DNA methylation and ncRNA also play an important role, which are often omitted in research as mechanisms associated with AD. Yu et al. noticed in their study that despite single biomarkers for AD having been determined on a genome-wide scale, the differential co-expression in gene pairs between regions and interactions with other types of cellular molecules, particularly non-coding ncRNAs, are often overlooked in studies investigating the underlying mechanisms associated with AD.[49]

Conclusions

Meta-analysis and comparative analysis of the gene complex responsible for AD

Looking at multiple genes together rather than analyzing them individually, may improve identification of AD risk alleles.[4,50] Moreover, there may be multiple sufficient risk sets for AD. LOAD is highly polygenic with 30 loci identified in human DNA by GWAS and early whole exome sequencing (WES) study.[51] Recent advances in technology and Internet development have led to new methods for studying various diseases called the "omics era." It allows the collection of large amounts of data and information at the molecular and protein level, along with the development of novel computational methods and the use of statistical tools that are able to analyzing and filtering such data, advances in the genotyping matrix, next generation sequencing (NGS), mass spectrometry technology and bioinformatics have enabled the simultaneous large-scale studies of thousands of genes, epigenetic factors, RNAs, metabolites and proteins, with the possibility of integrating many types of omics. All of these technological innovations have modified the approach to research on complex diseases, including Alzheimer's (AD). All above methods and GWAS, TWAS, and EWAS are a promising tool to study the relationship between several molecular pathways in AD and other diseases.[2,52]

In conclusion, to gain further understanding of the role of described genes in risk of AD scientists should have deeper sequencing approaches (Whole-Genome Sequencing -WGS) to identify variants in larger insertions and deletions and signals from non-coding regions of DNA. As of today, it is known that about 67 autosomal genes are strongly associated with LOAD. So far, there are no drugs aimed at alleviating the effects of polymorphisms and mutations in these genes.

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REVIEW PAPER

Michał Osuchowski [1(ADFG)], David Aebisher [2(FG)], Joanna Gustalik [1(AG)], Dorota Bartusik-Aebisher [3(FG)], Ewa Kaznowska [1(FG)]

The advancement of imaging in diagnosis of prostate cancer

[1] Department of Pathomorphology, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[2] Department of Photomedicine and Physical Chemistry, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[3] Department of Biochemistry and General Chemistry, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland

ABSTRACT

Introduction. Multiple imaging methods have been used to stage prostate carcinoma. Some of them are easily accessible, others very accurate. The advancements over many years have been taken under consideration and now every imaging method has a specific role in the diagnosis of this malignancy.

Aim. There are over 1,100,000 cases of prostate carcinoma diagnosed every year around the world. Imaging examinations have to be introduced to accurately stage, and therefore properly treat this disease. This review concentrates on advantages and disadvantages of different imaging methods.

Material and methods. The literature search was performed.

Results. Imaging methods serve specific goals. TRUS is recommended for acquiring biopsy specimen due to high accessibility and low cost of the examination.

Conclusion. The best tool for staging prostate carcinoma and finding suspicious lesions when attempting second biopsy is mpMRI or bpMRI.

Keywords. MRI, PET, prostate carcinoma

Imaging

Prostate carcinoma is the second most frequent malignant tumor diagnosed in the male population worldwide.[1-3] North America and Europe are the regions with the highest number of newly diagnosed cases.[4] Studies prove that this neoplasm will affect 1 in every 6 men during their lifetime.[5,6] Pathologists in the United States diagnose prostate carcinoma in over 80% of patients in their 70's upon post mortem tissue examiantion.[7,8] Therefore, diagnosing a clinically relevant disease that requires treatment is a priority.[9] Physical examination and Prostate Specific Antigen (PSA) serum levels are usually the first tests when diagnosing prostate carcinoma.[10] PSA serum levels can be increased not only by

Corresponding author: David Aebisher, email: daebisher@ur.edu.pl

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cancer, but also by other diseases or factors, for example an inflammation of the prostatic gland.[11] Other tools have to be introduced to properly identify and stage prostate carcinoma.[10,12]

The first imaging method used to evaluate the prostatic gland lesions was the transrectal ultrasound (TRUS). It provided good-quality images of the organ because a high-frequency probe was placed in the rectum close to the prostate.[13-15] Initially TRUS of prostate was performed to evaluate for prostatic disease including prostate cancer, benign prostatic hyperplasia (BPH), prostatitis, prostatic abscess, and prostatic calculi.[16-19] Currently, it is mainly used for TRUS guided core biopsies. [10,20,21] Most cases of prostate carcinoma are hypoechoic or hyperechoic on ultrasound imaging but even up to 40% are isoechoic. This fact significantly limits the role of TRUS in detection of this malignancy.[23,24] Furthermore hypoechoic areas within the peripheral zone can also be seen in benign processes.[25] A significant part of TRUS called volume assessment of the prostate is useful in planning treatment with brachytherapy or cryotherapy.[16] Most recent advances in ultrasound imaging include micro-ultrasound systems that introduce 29 MHz probe to assess the risk of prostatic carcinoma and enable real-time targeted biopsies. New methods allow for decreasing clinically-insignificant cancer diagnoses and detecting high risk disease early.[26-28] Computed tomography scans are used to identify metastases but not for staging the disease.[9,10] MRI is the most accurate and reliable non-invasive method when diagnosing prostate carcinoma.[29] It has been suggested that magnetic resonance spectroscopic imaging (MRS) is even capable of determining the grade of prostate carcinoma.[30] The currently used 3-Tesla MRI offers high resolution view that is capable of identifying small foci of cancer that are not visible on TRUS.[31-34] The combination of basic T_1-weighted and T_2-weighted images and more advanced dynamic contrast-enhanced (DCE) or diffusion-weighted (DWI) imaging is called multiparametric MRI (mpMRI). This method has the highest negative predictive value of all imaging techniques.[12,33] The mpMRI is currently being used mostly to diagnose patients with high risk of prostate carcinoma and a negative result in first biopsy. It helps to identify the most suspicious areas in order to guide the second biopsy. This helps in obtaining the most representative tissue sample.[35,36] In spite of all of the advantages of mpMRI, it is not the primary imaging method for biopsy guidance.[10] This is the case for several reasons. The mpMRI's availability is limited, it's expensive and has low inter-reader reproducibility.[37,38] Some authors suggest that these problems could be at least partially solved by biparametric MRI (bpMRI). Reducing cost, time, and contrast exposure is achieved by eliminating the DCE phase of the imaging without forfeiting valuable diagnostic information. Both bpMRI and mpMRI offer similar cancer detection rates for clinically significant prostate carcinoma.[39,40]

When evaluating the stage of prostate carcinoma hybrid imaging devices in the form of single-photon emission CT/CT gamma cameras (SPECT) or positron emission tomography/CT cameras (PET) are very useful. These methods are designed to diagnose metastases.[41] With SPECT imaging bone metastases can be detected with very high sensitivity and specificity (over 79% and 82% respectively).[42] PET imaging using ^{11}C-choline or ^{18}F-choline as contrast agents can be used to diagnose lymph node and bone metastases. For the latter, sensitivity is at 100% and specificity is around 86%.[18] Due to relatively low glucose absorption by prostate carcinoma, the use of FDG-PET imaging method is very limited. [22]

Conclusion

The advancements in imaging methods have allowed for accurate staging of prostate carcinoma when evaluating the clinically significant disease. This leads to more effective treatment and surveillance of patients with this malignancy. The mortality of patients with prostate carcinoma, second most frequent malignant tumor in men, is only at around 10% and diagnostic imaging is a big part of that success.[43]

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REVIEW PAPER

Katarzyna Błochowiak (ABDF), Paweł Aleksandrzak (BDG), Kacper Kropielnicki (BDG), Jakub Handschuh (BDG), Patrycja Pawlik (F), Anna Stanek (F)

Extraction of asymptomatic impacted third molars – a review

Department of Oral Surgery and Periodontology, Poznan University of Medical Sciences, Poznań, Poland

ABSTRACT

Introduction. The prophylactic extraction of asymptomatic wisdom teeth is defined as the surgical removal of wisdom teeth in the absence of local disease. Early extraction of asymptomatic third molars is considered beneficial to patients to prevent the risk of future pathology, and to minimize operative and postoperative risks. The second concept is watchful monitoring of asymptomatic wisdom teeth, adhering to specific indicators for their extraction.

Aim. The aim of this paper is to present and evaluate the indications and effects of prophylactic extraction of asymptomatic impacted third molars in adolescents and adults, compared with their retention and watchful monitoring.

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

Conclusion. There exist clear indications for the extraction of third molars which are associated with pathology. Prophylactic extractions of asymptomatic impacted third molars should be performed only before 20 years of age. In older age, asymptomatic third molars should be retained and watchfully monitored, and removed only in cases of evident clinical or radiological symptoms.

Keywords. impaction, surgery, third molars, wisdom teeth

Introduction

Third molar extractions are one of the most common dental surgical procedures, especially in young adults. Since many third molars require surgical removal, the costs associated with this procedure can be significant. Although the risks associated with third molar extractions are generally minor, like pain and swelling, some complications may be more serious, such as injury to the temporomandibular joint (TMJ), or permanent tongue or lip paraesthesia. Third molars do not always fulfill a functional role in the mouth, however, they are the most common teeth to be impacted. They can be asymptomatic for many years with a lack of indications for extraction. Conversely, third molars can be the source of many pathologies, including recurrent pericoronitis, development of follicular cysts and dentigerous tumours, deep caries in second and third molars, neuropathic pain, TMJ abnormalities, and periodontal pockets. Due to decisions regarding removal or retention of third molars often being made in late adolescence and early adulthood, understanding the risks and benefits of removal or retention during this time period is impor-

Corresponding author: Katarzyna Błochowiak, e-mail: kasia@naszdentysta.com.pl

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tant. There are two strategies for managing third molars. The first concept, prophylactic extraction of asymptomatic wisdom teeth, is defined as the surgical removal of wisdom teeth in the absence of local disease. Early extraction of asymptomatic third molars is considered beneficial to patients to prevent the risk of future pathology, and to minimize operative and postoperative risks. The second concept is watchful monitoring of asymptomatic wisdom teeth, adhering to specific indicators for their extraction.[1-10]

The aim of this paper is to present and evaluate the indications and effects of prophylactic extraction of asymptomatic impacted third molars in adolescents and adults, compared with their retention and watchful monitoring.

Indications for third molar extraction

The current clinical condition and the potential risk of future complications are the most important factors in choosing the appropriate strategy. Mandibular third molars have the greatest incidence of impaction with 33% of the population having at least one impacted tooth.[6] Third molars are frequently impacted due to skeletal insufficiency in the area of their eruption. Sagittal growth of the mandible finishes earlier than eruption of third molars, in many cases leading to impaction. Proper mandibular third molar eruption also depends on a favourable path of eruption. If the tooth bud is medially angulated during the initial stages of calcification and root development, this path of eruption will be unfavourable. Root angulation is also correlated with third molar impaction. Angulated roots are more common in impacted mandibular third molars, as compared to erupted mandibular third molars.[4-6] However, unfavourable prognosis of eruption and impaction of third molars does not always give clinical symptoms and local diseases. Indications for tooth extraction are based upon the recommendations of the American Association of Oral and Maxillofacial Surgeons (AAOMS), and include pericoronitis, operculitis, periodontal ligament damage and bone loss, adjacent tooth root resorption or caries, radiolucency caused by the impacted tooth, impaction of both third and adjacent second molars, unrestorable caries, overeruption, and a missing antagonistic tooth.[11] The prophylactic removal of asymptomatic impacted wisdom teeth is defined as the surgical removal of wisdom teeth in the absence of local disease. In most Western countries, the prophylactic extraction of asymptomatic third molars, either impacted or fully erupted, has long been considered as appropriate care. However, prophylactic extraction of asymptomatic wisdom teeth may lead to considerable postoperative complications. The prevalence of asymptomatic impacted third molars varies widely and is influenced by age, gender, and ethnicity. The low frequency of pathological changes related to impacted wisdom teeth has been used to promote a more cautious approach. Health risks and cost-effectiveness of the prophylactic removal of asymptomatic impacted wisdom teeth should play a more prominent role in the decision-making process. Moreover, as the costs of surgical extractions are significant, removal of asymptomatic impacted wisdom teeth that may remain disease-free indefinitely produces an unnecessary burden on healthcare resources. Prudent decision-making, with adherence to specific indicators for extraction, may reduce the number of surgical procedures by 60% or more.[12] The decision-making process regarding the prophylactic extraction of asymptomatic impacted wisdom teeth should be based on the patients' perspectives, values, and attitude.

One of the most relevant causes of prophylactic extractions of impacted third molars is the surgical extraction of asymptomatic wisdom teeth following orthodontic therapy to prevent late incisor crowding. The results of research are controversial and they do not correspond to each other. According to Mettes et al., it is not possible to predict whether a given adolescent participant will benefit from the extraction of impacted third molars with regard to late incisor crowding.[13] The length of the arch increases in some participants while in others the arch length decreases during the observation period. The length of the arch in the whole sample does not change differently on the extraction side when compared with the control side of the same patients. The extraction side has a more favourable development than the control side in 70% of cases; however, the control side has a more favourable development in 30%.[14] The other trial shows no significant difference between both groups. The conclusion drawn from this randomized prospective study is that removal of impacted third molars to reduce or prevent late incisor crowding cannot be justified. Despite a remarkable agreement regarding third molar prognosis, orthodontists and oral maxillofacial surgeons were unable to predict lower third molar eruption by examining a simple panoramic radiograph.[15] Moreover, the shortening of the arches is to some extent due to the pressure exerted by the erupting second molars. However, a reliable association between this event and third molars cannot be made until jaw growth and root development is completed. Mettes et al., compared extraction of asymptomatic wisdom teeth with their retention and only evaluated the effects on crowding after a 5 year follow-up. Three measurements of crowding were assessed: intercanine width (ICW), arch length (AL) and Little's irregularity index (LII), and the results were inconsistent.[13] The main limitation of these studies is a loss of future follow-up in the retention group of older aged patients. According to Friedman et al., third molars do not possess sufficient force to move other teeth. It is not possible for lower

third molars, which develop in the spongy interior cancellous tissue of bone with no firm support, to push 14 other teeth with roots implanted vertically, like the pegs of a picket fence, so that the incisors in the middle twist and overlap.[16] Similar results are presented in a study by Costa et al., where no significant differences were found in lower incisor crowding between patients from whom third molars were extracted, and from those on whom no intervention was performed; thus, the prophylactic extraction of third molars is not justified.[17] On the other hand, severe crowding is an indication for third molar extraction.[17] Furthermore, a crowding problem may be multifactorial in origin, and the third molar can be influential in individual cases.[14] Prophylactic extraction is an attempt to avoid or minimize periodontal morbidity associated with the retention of impacted third molars. The general conclusions from both retrospective and prospective clinical observations are that impacted third molars represent a serious periodontal hazard, which cannot be overcome by the usual procedures for pocket eradication, and delay in removal of impacted third molars beyond 25-30 years of age is detrimental to the periodontal health of the second molars.[14] Maxillary third molars are principally a source of future periodontal problems. Many studies have stressed the importance of prophylactic extraction of third molars to prevent periodontal pathology and the potential systemic health problems associated with periodontal disease.[18] The prophylactic extraction of asymptomatic third molars should be performed before the onset of symptoms. A common symptom of periodontal pathology is a pocket depth of 4 mm or deeper. Although these pockets in the second and third molar area may be influenced by the eruption status of the third molar, it is unclear whether a 4 mm pocket is always indicative of periodontal disease, as it is usually based on attachment loss and the presence of inflammation, rather than just pocket depth.[2] Previous studies reported that 38% of second molar distal sites with 4 mm or more pocket depths at baseline had an increase in pocket depth of 2 mm or more during the follow-up period of 2.2 years.[19] Prophylactic asymptomatic extraction of impacted third molars reduces probing pocket depths (PPD) and probing attachment levels (PAL).[20] Reduction of these indices positively correlates with clinical improvement. Young patients may benefit from early extraction of mandibular third molars, especially in the presence of certain cofactors.[20] Attachment loss is a more preferred periodontal pathology symptom than deep periodontal pockets. The average rate of attachment loss at distal sites of second molars was minimal over a 2-year period, regardless of third molar retention or extraction.[2]

The dentist's management of third molars commonly hinges on identifying the presence of symptoms or diseases that are clearly attributable to the third molar. Dodson developed a useful guide that serves as a systematic and unambiguous way to classify third molars. According to Dodson, patients' symptoms are designated as present and attributable to the third molar (Sx+) or as absent (Sx-). In addition, clinical or radiographic evidence of disease is evaluated and designated as present (D+) or absent (D-).[21]

Table 1. Dodson's classification

Group	Symptoms attributable to third molars (Sx)	Clinical or radiographic evidence of disease (D)
Group A: pericoronitis, dental caries, infection (fascial space infection, pulp necrosis)	Yes	Yes
Group B: myofascial or deafferentation pain (atypical)	Yes	No
Group C: periodontitis, periodontal attachment loss, coronal caries, cyst or tumour associated with the tooth	No	Yes
Group D: non-functional (unopposed and soon to supra-erupt), orthodontic indications, planned orthognathic surgery, removable prosthetics	No	No

This classification may change the description of asymptomatic third molars and indications for their extraction. The absence of symptoms which are common indications for third molar extractions, does not always reflect the true absence of disease.[21] Data presented in this classification is not sufficient to refute or support prophylactic extraction of third molars in group D versus active surveillance. Active surveillance, a prescribed program of follow-up and reassessment at regular intervals, is recommended for retained third molars, rather than waiting for the onset of symptoms to initiate a follow-up.[21] Ventä et al., recommends the preventive extraction of third molars at a young age in 3 groups of teeth: partially impacted teeth in the horizontal position, partially erupted teeth in the vertical position, and incomplete roots growing close to the mandibular canal. About one fourth of retained and disease-free third molars need to be removed preventively at a young age, whereas the rest should be treated according to signs and symptoms.[22] The estimated risk of complications, inclination of molars, age, degree of impaction, and patient sex, in decreasing order, are the main factors influencing the decision to extract third molars. The surgical experience of the dental professional does not seem to influence treatment decision.[23]

One of the most relevant factors for prophylactic extraction of third molars is a high incidence of pathology. However, this incidence is the same as for appendicitis (10%) and cholecystytis (12%), yet prophylactic appendectomies and cholecystectomies are

not the standard of care. No more than 12% of impacted teeth have an associated pathology.[16] Moreover, the risk of pathology in impacted third molars does not increase with age. The most severe pathologies related to third molars are dentigerous tumours and follicular cysts. The prevalence of cyst and tumour development around mandibular molars ranges between 2% and 6.2% in the long term.[24] On the contrary, histologically detected pathologic changes in the follicles of impacted third molars are found in 23% of asymptomatic third molars.[25] Simsek et al., detected cystic changes in 10% and inflammatory changes in 62% of extracted asymptomatic lower third molars.[26] There is no relation between the angular position of the tooth and pathologic changes.[25,26] Asymptomatic third molars should be actively monitored. Conversely, cyst formation is determined by age and tooth development. The occurrence of squamous metaplasia is greater in patients older than 20 years of age, demonstrating that the prevalence of squamous metaplasia increases with age. Moreover, a significant association is also observed between inflammation and squamous metaplasia.[27] These two factors support the argument for early extraction of impacted third molars. Pericoronal radiolucencies wider than 2.5 mm seem to dysregulate cell death and increase anti-apoptotic bcl-2 protein activity, which increases the likelihood of pathological changes arising in the follicle of third molars.[24] A study of more than 1756 patients who had retained more than 2000 mandibular impacted teeth for an average of 27 years, found that only 0.81% experienced cystic formation.[16] According to the same authors, even a single episode of pericoronitis is not a reason to extract a third molar, and should only be considered if the problem fails to respond to conservative treatment or recurs. Overall, 20% of cases consist of pathologies and pericoronitis associated with impacted third molars.[16] Most discomfort experienced during the eruption of wisdom teeth is equivalent to teething and disappears on full eruption. Most infections of the gum tissue around the erupting or partially erupted teeth can be prevented by good oral hygiene. Infection occurs in fewer than 10% of third molars, most of which can be cured with antibiotics, oral rinsing, or removal of excess tissue around the tooth, without requiring extraction of the tooth itself. One of the suggested indications for removal of third molars is the suspicion of possible pathology in second molars. Current literature reports a low prevalence of second molar external resorption (0.3 to 7%), although this percentage can be four times higher if, instead of analyzing panoramic radiographs, Cone Beam Computed Tomography (CBCT) is used. Second molar resorption is selected by more than 11% of the clinicians as an indication to extract third molars.[24] Additionally, impacted third molars and distal surfaces of second molars are prone to caries. According to Nunn et al., the presence of a third molar that is soft tissue impacted leads to a 4.88-fold increase in the risk of incidence of second molar pathology. Having an erupted or "bony" impacted third molar increases the risk of incidence for second molar pathology by 1.74 and 2.16, respectively. The retention of third molars is associated with increased risk of second molar pathology in middle aged and older adult men.[28] According to Huang et al., the incidence of caries on the distal surface of the second molars is less than 1%. Caries rate for third molars is approximately 3.3% on the occlusal surfaces.[2] The incidence of caries on the distal surfaces of second molars is extremely low, whether third molars are extracted or retained. In patients who returned for a 2-year follow-up examination, fewer than 0.5% of surfaces displayed evidence of caries overall.[2] This complication depends on the depth of impaction and position of the impacted tooth. The probability of developing caries in the distal aspect of the second molars increases when the angulation between the third and the second molars is between 43° and 71°, or if the distance between the cementoenamel junction of the two teeth is between 3 and 10 mm.[24] Similar rates of angulation as an indication for prophylactic extraction are suggested by Srivastava et al.[29] Fernandes et al., analyzed how many third molars survived 1 year of study period symptom-free. After 1 year, only 37 teeth had been extracted from the 676 teeth examined. 94.53% of all teeth survived the study period. After 1 year, 562 teeth (81.13%) of all 676 teeth observed survived the study period symptom-free. About 114 teeth (16.87%) developed some form of symptoms over the study period. It is very interesting to note that the development of symptoms is not necessarily translated into extraction, and unfortunately a small amount of teeth (1.48%) were extracted without any symptoms recorded by the clinician or any symptoms that could be remembered by the patients themselves.[3]

There are differences in management with third molars between general dentists and oral surgeons. Oral surgeons recommend third molar extraction more often than general dentists. Another noteworthy aspect of third molar management is patient adherence to the dental professional's recommendation. Although many patients are referred for third molar extraction by their general dentist or orthodontist, studies usually only focus on patients who present to oral surgery clinics. Adolescent patients and their parents may or may not follow their dentist's recommendation to retain or extract third molars.[30,31] In the study of Cunha-Cruz et al., the main reasons for recommending extraction were to prevent future problems (79%), unfavourable third molar orientation, and third molars that were unlikely to erupt in the dentist's opinion (57%). The least common reasons for recommending third molar extraction are

pericoronitis, periodontal concerns, dental caries and other pathologies. Dentists recommended retention and monitoring in 46% of participants. The main reason for recommending retention was that it was too early to decide (73%), followed by favourable eruption (26%), and fully erupted third molars (16%).[31] When dentists recommended extraction, 55% of participants adhered to this recommendation during follow-up, and the main reason was the availability of insurance. General dentists frequently recommended extraction of third molars for reasons not related to symptoms or pathology, but rather to prevent future problems.[31] Although evidence on the benefits of asymptomatic third molar extraction is conflicting, dentists recommend prophylactic extraction on the basis of unfavourable prognosis and for prevention of future possible problems. According to general dentists, monitoring of asymptomatic third molars is a more cost-effective strategy for the management of third molars.[31]

In previous research, many patients reported pain and swelling associated with third molars and wanted third molars extracted to prevent a recurrence of these symptoms, which decreased their quality of life. The severity of any pre-surgical morbidity may help clinicians and patients select treatment alternatives in circumstances where clinical indicators alone do not provide a clear-cut indication of whether to proceed with surgery. Patients whose quality of life is adversely affected by pre-surgical conditions may elect to have surgery, even when clinical criteria suggests that surgery and conservative management could be equally effective. The concept of prophylactic extraction of third molars must consider the patient's quality of life, by aiming to reduce the first symptoms or future possible recurrent pain or swelling. In the study of Slade et al., one third of patients said they were seeking third molar surgery because of current or previous symptoms of pain or swelling, and 17% reported one or more of the 12 non-pain-specific Oral Health Impact Profile (OHIP) questionnaire items.[32] A contemporary view is that health involves more than the absence of disease. The quality of life, social, psychological aspects, and interaction are now accepted as an integral part of overall health. These findings showed that if patients had third molar symptoms of pain and swelling sufficient to prompt them to seek surgery, their quality of life is adversely affected. The fear for future pain or swelling may be an important indication for prophylactic extraction of asymptomatic third molars.[32] It is worth noting that when offered the choice of retention or extraction, most patients (60%) with asymptomatic, disease-free third molars elected for extraction. When symptoms or diseases related to third molars are present, more than 95% of patients chose extraction as the preferred treatment.[33]

Postoperative complications

Extraction of third molars is associated with postoperative complications, with reported rates ranging between 6.9-30.6%. According to Schwartz-Arad et al., the total complication rate of third molar extractions was 16.9%.[11] Their development is conditioned by local and general factors including tooth position, age and health status of the patient, knowledge and experience of the surgeon and surgical equipment, surgical technique, and inappropriate irrigation during surgery.[5] The complications associated with removal of impacted teeth might be more serious when compared with the same observed complications in younger patients. Zhang et al., detected a higher incidence of postoperative complications in the group of patients above 23 years old with mature teeth with closed apical foramen, than in patients below 23 years old with immature teeth without a closed apical foramen.[34] Immediate complications include pain, trismus, swelling, dysphagia, while delayed complications include bleeding, dry socket, wound dehiscence, delayed wound healing, infections, periodontal pocketing, and nerve injury.[5,11] The rates of frequency of swelling and mild pain after extraction of impacted third molars were 10% and 40%, respectively.[5] Difficult extraction of impacted third molars may trigger periodontal pocketing distal to the second molars. According to Coleman et al., probing sites after 6 months post-extraction remain unchanged and extraction of the impacted maxillary third molar does not result in significant periodontal defects on the distal aspect of the adjacent second molar. Moreover, in many cases it results in an improvement of the probing depths of these teeth.[35] Postoperative complications depend on the degree of impaction. Partially impacted teeth show the highest degree of complications. The incidence of postoperative dry socket in the presence of caries or pericoronitis is reported as 21.9%, compared to 7.1% without any symptoms.[11] Preoperative pericoronal inflammation is also a risk factor for complications after third molar surgery.[4,36] This further supports the idea of prophylactic extraction of asymptomatic third molars to eliminate possible, recurrent complications associated with these teeth. These complications are also age-dependent. The lowest complication rate was observed in the 10-to-18-year age group (4.8%) whereas in the >36-year age group, the complication rate was more than four times higher. Reduction in complications in the 10-to-18-years group highlights the importance of extracting third molars at an early stage, prior to the completion of root formation.[4,11] Younger patients are less prone to postoperative complications.[19] Reduction of dry socket, pain and swelling in adolescents and young adults are indications for prophylactic extractions of asymptomatic third molars. Conversely, partially impacted teeth had a higher incidence of postoperative lingual numbness (14.9%), compared to fully impacted teeth (9.7%).[7] Old-

er age also increases the risk of temporary lingual and inferior alveolar nerve damage.[34,37] Patients in the youngest age group were found to have a lower risk of an extended operation time than older patients. Shorter time required for extraction in younger patients was likely associated with the structure of bone and the level of tooth development.[37] The higher risk of postoperative complications in elderly patients might be due to the different techniques of tooth extraction used in the elderly, because of the higher density of bone. Osteoporotic or sclerotic bone, dental ankylosis, use of various drugs for coagulation may all predispose to postoperative complications and more difficult surgical extraction of third molars in a more advanced age.[6] Another explanation may be that erupted molars in older patients have been used for mastication and are therefore more tightly connected to the alveolar bone by the periodontal ligament.[5] Many oral surgeons recommend prophylactic extractions of asymptomatic third molars at a younger age because of the simpler technique of extraction, shorter procedure time and lower risk of postoperative complications. Most postoperative complications such as dry socket, swelling, trismus, pain and delayed-onset infections (DOI) are less severe in younger patients after prophylactic extraction of asymptomatic third molars. In contrast, previous research reported that the rates of lip or tongue paraesthesia was significantly higher in patients who underwent third molar extraction. Moreover, these effects lasted longer than the immediate postsurgical period. Paraesthesia has been reported to occur in about 1% to 6% of patients undergoing third molar extraction.[2] Occurrence of these complications depends on third molar orientation, depth of impaction and anatomical relationships between third molars and the mandibular canal. Deep impaction of third molars significantly increases surgical difficulties and the risk of inferior alveolar or lingual nerve damage. In our opinion, it is the most severe and possibly permanent postsurgical complication, and should be taken into consideration when making the decision to extract asymptomatic impacted third molars.

TMJ symptoms are often associated with third molar eruption or impaction and they are indications for third molar removal. It is worth noting that the rate of TMJ symptoms reported by patients who had undergone a third molar extraction was much higher than expected. More than 30% of patients presented with either joint pain or muscular pain.[2] A growing body of evidence has indicated that third molar extractions may result in TMJ symptoms. Prophylactic extractions of third molars do not relieve TMJ symptoms and signs such as clicking, jaw pain on wide opening, pain in temples, but may even cause or exacerbate such symptoms.[2]

The second group of postoperative complications is delayed-onset infections (DOI) after mandibular third-molar extractions. They are rare and are characterized by swelling, usually with a purulent discharge at the extraction site, developing approximately a month after surgery. The incidence reported in the literature ranges between 0.5% and 1.8%.[4] According to Brunello et al., DOI incidence was reported in 3.7% and included dehiscence, swelling, trismus, exudate, purulent discharge, lymph node enlargement, and pain on palpation. The median time elapsing from the extraction to the DOI was 35 days. Younger age and longer surgical procedures seemed to be more often associated with this complication.[4]

The most important issue in case of watchful monitoring of asymptomatic third molars is the time interval of monitoring. According to Huang et al., a 2-year monitoring period is most recommended because when third molars were not extracted, considerable eruption occurred during this period.[2] A similar period of active surveillance is recommended by Dodson et al.[38] Active surveillance is characterized as a regularly scheduled set of follow-up visits that include both clinical and radiographic examinations. 50% of all third molars that were classified as partially erupted at the time of enrolment were classified as fully erupted at the end of the study. Even 18% of teeth classified as soft tissue impactions at baseline were considered fully erupted 2 years later.[2,39] Venta et al., reported on eruption for patients in their early 20s.[39]

There is still an ongoing disagreement regarding the prophylactic extraction of asymptomatic impacted wisdom teeth. There were no reliable methods to predict future pathologies related to impacted third molars. The limited information on the prevalence of pathology related to third molars in older patients suggests that prophylactic extraction of all impacted third molars before adulthood may not be justified. Longer follow-up periods are required to obtain more concrete data. Other diagnostic methods that are also indicated for third molar eruption prediction should be investigated, such as longitudinal radiographs or 3D images. Most applied techniques for the prediction of third molar impaction or eruption have involved the use of panoramic radiographs, lateral and postero-anterior cephalograms, focusing essentially on the relationship between the third molar and the space available in the retromolar area.[40] Additionally, the decision about whether to recommend extraction or retention of asymptomatic wisdom teeth may also be influenced by factors such as cost and possible professional liability.

Conclusion

The decision to extract pathology-free third molars should be based on the risks and benefits of extraction, as well as the consequences of their retention in the mouth. The patient should be involved in the decision and informed of all possible options. There exist clear indications for the extraction of third molars which are

associated with pathology. Prophylactic extractions of asymptomatic impacted third molars should be performed only before 20 years of age. In older age, asymptomatic third molars should be retained and watchfully monitored, and removed only in cases of evident clinical or radiological symptoms. Active surveillance allows the dental professional to detect possible pathology before the onset of disease. The detection of these signs is an indication for tooth extraction.

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REVIEW PAPER

Piotr Bar [1(ADFG)], Sabina Galiniak [2(FG)], Dorota Bartusik-Aebisher [2(FG)], Rafał Filip [3(FG)], David Aebisher [4(FG)]

Infliximab in therapy of inflammatory bowels diseases

[1] Clinical Department of Pathomorphology, Clinical Hospital No. 2, Rzeszów, Poland
[2] Department of Biochemistry and General Chemistry, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[3] Department of Gastroenterology, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[4] Department of Photomedicine and Physical Chemistry, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland

ABSTRACT

Introduction. Infliximab is a monoclonal antibody that acts against tumor necrosis factor TNF-α. The drug is used in the treatment of autoimmune diseases.

Aim. This article reviewed the efficacy and safety of infliximab for the treatment in severe ulcerative colitis. This review included studies that evaluated the clinical use of infliximab.

Material and methods. This meta-analysis was performed according to systematic literature search of three major bibliographic databases (Scopus, PubMed, and Cochran).

Results. Infliximab has been approved by the US Food and Drug Administration (FDA) as a medicine to treat Leśniowski and Crohn's disease, ulcerative colitis, psoriasis, psoriatic arthritis, ankylosing spondylitis, and rheumatoid arthritis. However, further trials are required to compare other parameters of efficacy such as the clinical response with infliximab.

Conclusion. In patients suffering from Crohn's disease or ulcerative colitis under infliximab maintenance therapy, sustained good trough levels are associated with: better response and remission rates, more mucosal healing and less loss of response.

Keywords. infliximab, TNFα, ulcerative colitis

Introduction

There is still a need to develop new effective medications for the treatment of ulcerative colitis, particularly for patients who are intolerant or resistant to first line therapies.[1-5] Current pharmacotherapy for inflammatory bowel diseases are: derivatives of 5-aminosalicylic acid; glucocorticoids; purine analogs; antibiotics, metronidazole, quinolones and biological treatment anti-TNFα antibodies (infliximab, adalimumab, certolizumab). Infliximab is a chimeric immunoglobulin G1 (IgG1κ monoclonal antibody), monoclonal antibody which contains a human constant region and a mouse-derived murine variable region. Infliximab (molecular weight of approximately 149.1 kilodaltons) is specific for hu-

Corresponding author: David Aebisher, email: daebisher@ur.edu.pl

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

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man tumor necrosis factoralpha (TNFα).[6-8] Infliximab is clinically used as lyophilized concentrate for injection and get approval in 1998. The drug cannot be administered orally, because the digestive system destroys the active part of the drug. Infliximab is administered by intravenous infusion.[9-12] Tumor necrosis factor α; TNF-α, is one of the main mediators in inflammatory processes and plays an important role in the pathogenesis of many chronic inflammatory diseases - rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis or juvenile idiopathic arthritis.[13-17] The introduction of anti-TNF-α drugs into clinical practice has opened a new era in the treatment of chronic inflammatory diseases.[18-23]

Material and methods

Major bibliographic databases (Scopus, PubMed, and Cochran) were searched for the newest information about infliximab.

Results

A group of biological drugs that are known as TNF antagonists include human anti-TNF-α (adalimumab), chimerized, mouse-human anti-TNF-α (infliximab) antibodies, as well as fusion proteins that competitively bind to the TNF-membrane receptor α (etanercept).[24-27] TNF promotes the inflammatory response in rheumatoid arthritits, ankylosing spondylitis, Crohn's disease, ulcerative colitis and psoriasis. Inflammatory Bowel Disease (IBD) Infliximab neutralizes the biological activity of TNFα by binding to the soluble and transmembrane forms of TNFα and inhibits binding of TNFα with its receptors. Anti-TNF drugs such as infliximab also induce the formation of anticardiolipin antibodies (aCL).[28-32] Both of these drugs have approximately similar capacity to induce these antibodies. Inflammatory response has been shown to be ineffective in patients with aCL. Infliximab is approved for severe cases of rheumatoid arthritis, together with methotrexate, for pronounced psoriasis and psoriasis-arthritis, ankylosing spondylitis as well as for chronic inflammatory bowel disease.[33-43]

Generally, it is advantageous that infliximab be dosed with concomitant methotrexate to inhibit the formation of antidrug antibodies.[32-35] It is also thought that the concurrent dosing of methotrexate during the study reduces such immunogenicity. Infliximab has negative side effects, some are life-threatening, they are common to all drugs in the immunosuppressive class of TNF. Some of the most severe side effects are: serious infections, reactivation of hepatitis B reactivation of tuberculosis, lethal liver lymphoma (usually only in combination with 6-mercaptopurine), lupus, demyelination of the central nervous system, psoriasis and skin changes and new cases of vitiligo. Studies in both psoriatic arthritis and rheumatoid arthritis have established better patient outcomes using combination therapies with methotrexate. Lower doses of TNF-α antagonists are also more cost-effective for the patient. Infliximab has been associated with hepatosplenic T-cell lymphoma in inflammatory bowel disease patients treated concurrently with azathioprine. Thus, caution should be taken in using combination treatment and should not be first line because larger clinical trials are needed. Patients receiving infliximab are more susceptible to serious infections, including mycobacterial infections. Concomitant treatment with glucocorticoids was the only independent susceptibility factor for infections in patients with inflammatory bowel disease treated with infliximab.

Table. 1 Dose of infliximab and disease[33-43]

Disease	Dose
Crohn's Disease	5 mg/kg at 0, 2 and 6 weeks, then every 8 weeks
Pediatric Crohn's Disease	5 mg/kg at 0, 2 and 6 weeks, then every 8 weeks.
Ulcerative Colitis	5 mg/kg at 0, 2 and 6 weeks, then every 8 weeks
Pediatric Ulcerative Colitis:	5 mg/kg at 0, 2 and 6 weeks, then every 8 weeks. Rheumatoid Arthritis: In conjunction with methotrexate
Rheumatoid Arthritis	3 mg/kg at 0, 2 and 6 weeks, then every 8 weeks. Some patients may benefit from increasing the dose up to 10 mg/kg or treating as often as every 4 weeks
Ankylosing Spondylitis	mg/kg at 0, 2 and 6 weeks, then every 6 weeks. Psoriatic Arthritis and Plaque Psoriasis: 5 mg/kg at 0, 2 and 6 weeks, then every 8 weeks

Conclusion

In patients suffering from Crohn's disease or ulcerative colitis under infliximab maintenance therapy, sustained good trough levels are associated with: better response and remission rates, more mucosal healing and less loss of response.

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REVIEW PAPER

Hanna Czajka [1,2(ABCFG)]

Safety and efficacy of vaccinations in patients from high-risk groups: new challenges in the era of vaccine hesitancy

[1] Faculty of Medicine, Rzeszow University, Rzeszów, Poland
[2] St. Louis Regional Specialized Children's Hospital, Kraków, Poland

ABSTRACT

Introduction. Vaccinations are one of the most effective medical interventions that protect people against infectious diseases. It should be noted that a new vaccine licensing is always preceded by clinical trials assessing its safety and efficacy. Anti-vaccine propaganda carried out by vaccination opponents has become an international problem with a global reach.

Aim. To review the literature on vaccinations of patients from high-risk groups.

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

Results. High-risk groups in the paediatric population include pre-term born infants, patients after stem cell transplantations, children with allergies and other chronic diseases. Vaccinations in the examined groups are generally safe and are an effective method of preventing infections.

Conclusion. At a time when the level of vaccine skepticism is high and the epidemiological situation of many diseases is unstable, patients who are more susceptible to infection are particularly endangered. High level of knowledge of health care professionals and their personal positive attitude towards vaccinations are important for improving the vaccination coverage rates. In the light of measles epidemic outbreaks and an almost geometric increase in the number of pertussis cases noted recently, actions are needed to achieve herd immunity.

Keywords. allergy, immunogenicity, preterm, safety, transplantation, vaccination, vaccine hesitancy

Introduction

Vaccinations are one of the most effective medical interventions that protect people against infectious diseases. Many of these diseases posed a significant threat to health or life of children and adults just a few decades ago. Immunization procedures are considered to be expensive, and European Union member states spend on average 3% of their health budgets on protection against infectious diseases by vaccinations. Twenty different vaccines are currently being used in vaccination schedules in Europe, and a number of further new or improved vaccines are subject to advanced clinical trials.[1] It should be noted that vaccine safety and efficacy is always demonstrated during clinical tri-

Corresponding author: Hanna Czajka, e-mail: hanna.czajka@onet.pl

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als conducted under ideal conditions prior to licensing, but also after the introduction of a new vaccine. This applies to both monovalent and multivalent vaccines. In addition to the assessment of the effectiveness and safety of individual preparations, the design of national vaccination schedules is also important. It must ensure maximally simplified and safe co-administration of vaccines in order to reduce the risk of adverse events that may be caused by the interaction of simultaneously administered antigens. Nevertheless, since the very beginning the use of vaccines is accompanied by a large group of sceptics and determined opponents. With widespread and unlimited access to various, often unreliable sources of information, anti-vaccine propaganda carried out by opponents of vaccination has become an international problem with a global reach.[2] The false data and conclusions spread by those people or institutions are based on non-scientific argumentation. The problem manifests itself among others in an increase in the number of parents refusing to immunize their children or unjustifiably postponing the administration of vaccines. One of the most frequently attacked vaccines used in the pediatric population is the measles, mumps and rubella (MMR) vaccine. Information disseminated by vaccine opponents concern not only pediatric, but also adult population, which for example negatively influences the number of vaccinations against influenza carried out every year. In 2012 World Health Organization (WHO) together with the United Nations Children's Fund (UNICEF) established the Strategic Advisory Group of Experts (SAGE) on Vaccine Hesitancy. The purpose of this group is to recognize the reasons for vaccine hesitancy and to asses why children and adults are under-vaccinated or unvaccinated. Vaccine hesitancy results in the slowing of realization of vaccination schedules in the WHO Regions and individual countries even when the availability of appropriate vaccines is guaranteed.[3] The level of knowledge of health care professionals performing immunizations (doctors and nurses) and their personal attitude towards vaccinations are also important in the process of improving the rates of vaccine uptake.[4]

Aim

At a time when the level of vaccine skepticism is high and the epidemiological situation of many diseases is unstable, patients who are more susceptible to infection (high risk patients) are particularly endangered. Paying attention to the consequences of poor vaccine coverage including unreasonable delay of vaccinations has been the subject of my scientific and clinical interest for many years. The importance of safe administration of individual vaccines and the need to prepare recommendations for their use should also be stressed. The aim of the present work is to briefly review the literature available in this field.

Infectious Diseases Threats in Poland

In recent years the above-mentioned epidemiological instability concerned primarily measles. This highly contagious disease, until recently, had been the next eradication candidate after polio virus infections. A significant decrease in the incidence of measles between 2000 and 2015 (estimated at 75%) was a consequence of the introduction of routine vaccinations against this disease in a two-dose scheme. In Poland these vaccinations were started in 1975. In contrast to expectations, in 2015 an unprecedented growth in the number of measles cases was observed. WHO estimated the number of measles cases in 2015 at 9.7 million, the number of deaths from measles was estimated at over 130,000. At the same time, only 254,928 cases were reported to six regional WHO centers. This discrepancy is best illustrated by German data, where the number of measles cases reported to the mandatory notification system was three times lower compared to health insurance claim submissions by doctors.[5] In Poland, the number of cases of measles increased significantly at the end of 2018. Until October that year 148 cases were noted, in November 2018, 79 new cases were registered. A further 112 cases were observed in December, and eventually 339 people were diagnosed with measles in 2018 in Poland according to the official WHO report. As for 2019, as of 28/02/2019, 314 cases of this disease were reported in our country. In Poland, as in other European Union countries, measles affects mostly people who were not vaccinated against this disease (78%) or vaccinated with only one dose of MMR vaccine (16%).[6] Thus, in the current epidemiological situation, a significant increase in the number of people avoiding immunizations or refusing to have their children vaccinated is particularly alarming. The number of patients refusing vaccinations in Poland has increased more than ten-fold between 2010 and 2018 from 3,437 to 40,342 respectively.[7] This phenomenon is a direct threat to population immunity, which requires vaccine coverage rate at the level of at least 90%. In some provinces, the vaccination rate in children aged 2-3 years already does not reach this limit.

For many years, very low influenza vaccine coverage rates were noted in Poland, which applies also to the patients from the risk groups. Yearly updated global recommendations suggest that 75% of the population should be vaccinated against influenza every year which, however, is achieved only in a few countries. In Poland, in the age group above 50 years, less than 10% of people are vaccinated, and the total vaccine coverage in the 2016/2017 season has reached only 3.3%. Meanwhile, in the same season, over 2.5 million flu cases were registered in Poland in the group aged 0-14 years (over 5 million in the entire population), with approximately 10,000 hospitalizations and 48 deaths due to this disease.[8]

Recently a significant increase in the number of pertussis cases was also observed in Poland. A constant, almost geometric increase of incidence of this disease is noted in epidemiological reports of the National Institute of Public Health - National Institute of Hygiene in Warsaw. In 2014, 2,102 cases of pertussis were reported in Poland (incidence 5.46/100,000), in the following year this number has grown to 4,959 cases (incidence 12.89/100,000). In 2016, an increase by over 138% was reported (6,856 cases of pertussis, incidence 17.84/100,000). In these years the number of pertussis cases has grown almost fourfold (390%). Infectious diseases including pertussis are a significant threat to health and even to lives of infants. Pertussis infections are particularly dangerous to pre-term born children, including those with extremely low birth weight (ELBW) who are especially susceptible to infectious diseases. This is why early initiation of active prevention of infectious diseases (according to the chronological age of these children) is of great importance.[9] But epidemiological data show that vaccinations (also against pertussis) in the group of ELBW infants are started with a delay, which is associated with an increased risk of diseases that can be prevented in many cases.[10] Epidemiological data show that infants are particularly prone to bacterial or viral infections. Acute symptoms occurring in this age group usually require hospitalization and antibiotic therapy.[11] Pre-term born infants have a higher risk of hospitalization in the clinical course of pertussis compared to full term infants with normal body weight.[12] In addition to the immediate risk to health or life of a child, one should not forget about various complications of infectious diseases that can cause damage or permanent dysfunction of the nervous, circulatory, respiratory and muscular systems.[13,14]

Vaccinations in high-risk groups - pre-term born infants

In 2008, as a first in Poland, together with my colleagues from The Clinic of Neonatology at the Collegium Medicum of the Jagiellonian University, we proposed early vaccination of ELBW (<1000 g) infants born before 32 weeks of pregnancy. We postulated immunization of these children before their discharge, in the clinical setting.[15] This resulted from the observed unjustified delay in starting vaccinations in outpatient clinics after discharge from the neonatal intensive care units. Our proposal was based on studies of the American Academy of Pediatrics recommending starting vaccinations of preterm infants during hospitalization in neonatal wards.[16] Three years later – in 2011, I participated in the work of the Polish Expert Group, which issued recommendations concerning the vaccination of pre-term born infants in neonatal units. This recommendation was included in the Polish Vaccination Schedule for 2012. Thanks to these recommendations, it became possible to vaccinate pre-term born infants against diphtheria, tetanus, pertussis, poliomyelitis, Haemophilus influenzae type b and pneumococcal infections in addition to standard hepatitis B and tuberculosis vaccinations before their discharge from the neonatal intensive care units.[17] Despite the fact of developing the abovementioned recommendations a significant delay of vaccinations conducted by GPs after hospitalization is still observed in this group of patients. On the other hand only half of Polish neonatal units began administration of DTPa, IPV, Hib and PVC vaccines in pre-term infants in the clinical setting. It should be noted that the average duration of hospitalization of a pre-term born infant in Poland is 72 days.[18] Our own research shows that only 13% of 109 infants hospitalized between 2009 and 2014 in the Clinic of Neonatology at the University Hospital in Kraków (gestational age at birth 22-30 weeks, mean weight 953.4 g.) received the DTPa vaccine in accordance with the national vaccination schedule. Delays in the administration of vaccines in the studied group most often resulted from the unstable clinical condition of the patients. Despite a longer stay at the hospital, which was associated with various issues in the perinatal stage, the administration of the first dose of the DTPa vaccine took place sooner in the hospital setting than in the outpatient clinics (80 vs. 153.4 days).

In the analyzed group of patients vaccinated in the neonatological clinic 73.7% of children received a multivalent vaccine DTPa-IPV-Hib-HBV, which significantly improved the implementation of vaccinations, compared to children receiving vaccines purchased by the state. The use of standard vaccines used for mandatory vaccinations increases the number of required injections.

The assessment of the response to primary immunizations against hepatitis B, diphtheria, tetanus, pertussis in the group of ELBW infants showed the achievement of antibody concentration sufficient to provide adequate protection. The results of safety assessment were also positive – infants in the study group did not develop any adverse symptoms such as bradycardia, apnea, or drops in saturation.[19,20] Vaccinations in the risk group of pre-term born infants are not a frequent subject of prospective studies with a control group. During one of three multicenter clinical trials, carried out in 2015 in our clinic, the safety and efficacy of a conjugate vaccine against pneumococcal infections was assessed. The results obtained confirmed full safety and optimal efficacy of PCV13 vaccine in the group of pre-term born children. In the study conclusions the importance of protection against pneumococcal infections in this risk-group was emphasized together with the need of implementation of these vaccinations without delays.[21,22]

Vaccinations in high-risk groups – children with allergic diseases

Children with allergic diseases are often vaccinated with significant delays. In my everyday practice in the vaccination outpatient clinic in Kraków, the most frequent reason for consultations of patients with allergies is egg protein allergy. Parents of children allergic to egg proteins are afraid of allergic reactions after measles, mumps and rubella MMR vaccine administration. In view of the epidemiological situation outlined above, postponing the administration of the first dose of MMR vaccine should be qualified as a medical error. Since 2012, the recommendations of the American Academy of Pediatrics and the British recommendations indicate that MMR vaccine should be administered to children with an allergy to egg proteins in an outpatient setting, without special precautions. These recommendations have been fully approved by the World Allergy Organ since 2016. The analysis of a group of 138 patients with allergies consulted in our clinic because of the MMR vaccine administration postponement showed that in 101 cases (73.2%). The reason for immunization postponing was an allergy to hen egg protein. In this group the average delay in performing these vaccinations has reached 12.3 months. Vaccinations performed in our outpatient clinic, extended period of observation after the vaccine administration, as well as monitoring of the post-vaccination period demonstrated full safety MMR vaccination in the study group. There was no need for conducting any additional medical consultations or interventions.[23]

Vaccinations in high-risk groups - children after bone marrow stem cell transplantation

Attention should be paid to the particular importance of vaccination in reducing the risk of infectious diseases among patients in the early post-transplant period. In 2007-2010 in our Clinic we carried out medical qualification and vaccinations of children and adolescents referred to us by the Center for Transplantation of the Children's University Hospital in Kraków after autologous and allogeneic hematopoietic stem cell transplantations. In our hospital, we evaluated the seroprotection of 38 patients both before and after vaccination against diphtheria, tetanus, Haemophilus influenzae type b and against hepatitis B (HBV). The results of observation of these patients confirmed the efficacy and safety of performed vaccinations.[24,25]

The heterogeneity of this group is important to note, being the result of different indications for transplantation and various courses of the period after the transplantation.

For the aforementioned reasons, despite the recommendations to start immunization as soon as possible (3-6 months after autologous transplantation and 6-12 months after allogeneic transplantation), only a few patients started vaccinations according to the study protocol. The average time of the vaccination after auto-HSCT was 29 months (6-67 months) and 13 months after allo-HSCT (8-33 months). Parents of the patients even after being informed about the importance of the prevention of infectious diseases for their children's health both in the transplantation center and at the vaccination outpatient clinic were not fully convinced. It was due to some information of unknown origin that reached them from other, external sources and undermined their trust in vaccination of their children. As a result, five children did not receive the prescribed vaccines. Vaccinations were carried out in accordance with the European Group for Blood and Marrow Transplantation guidelines, which resulted from the lack of appropriate national recommendations.[26,27]

Vaccination safety

In each group of patients with an increased risk of developing infectious diseases vaccination recommendations include vaccination against measles, mumps and rubella (MMR) and against varicella (VZV). Immunosuppressed patients should always meet the conditions for the safe use of live vaccines prior to the vaccine administration. In order to maximally simplify the implementation of vaccination schedules and to ensure on-time vaccine administration, multi-component preparations for the pediatric population are created. Preparations created for this purpose are the 6-component DTPa-IPV-Hib-HBV vaccine and the 4-component vaccine against measles, mumps, rubella and chickenpox MMRV.

In view of high incidence of varicella in many countries with simultaneous occurrence of measles epidemic outbreaks concomitant use of two doses of MMR and VZV vaccine is required.[24] To simplify the vaccination schedule and to shorten the time of achieving protection MMRV vaccine was developed.[25] The safety analysis of its application, based on 8 clinical trials, showed that the administration of the first dose of MMRV, compared to separate administration of MMR and VZV vaccines, results in statistically more frequent occurrence of elevated body temperature within 15 days after MMRV administration.[28] This difference was not observed when MMRV vaccine was used as the second dose. These results led to conclusions and recommendations that the MMRV vaccine should be used as a second dose after first administering MMR and VZV vaccines separately.[29–32]

The safety of vaccinations is a priority task when creating vaccination schedules, but also during their implementation. The medical qualification for vaccination of patients who have previously had an adverse event after vaccine administration is a particular chal-

lenge. At the beginning of the 21st century, the lower percentage of infants received diphtheria, tetanus and pertussis (DTP) vaccine with acellular pertussis component. More frequent cases of hypotonic-hyporeactive syndrome (HHE) after vaccination with a full cellular component of pertussis were noted at that time. A group of 49 infants with a confirmed onset of HHE syndrome were given a DTPa vaccine to complete the vaccination schedule. No adverse reactions were observed. A further analysis of patients history, diagnoses and referrals to the vaccination outpatient clinic revealed poor level of knowledge concerning HHE as an adverse post-vaccination reaction among pediatricians as well as GPs .[33]

Vaccinations belong to everyday, routine practice of general practitioners. Hence, knowledge of current recommendations, contraindications to vaccination and adverse post-vaccination reactions is necessary for the proper implementation of the vaccination schedules and building confidence in vaccination among parents of our patients. According to CBOS research from 2017, the most common reason for refusing vaccinations was the fear of adverse events caused by vaccines. Therefore, better education of parents / guardians of children and providing full, reliable information by health care professionals is necessary. Improvement in the medical staff training is also crucial to meet these expectations. It is advisable to extend education programs at the level of studies of all faculties whose graduates may have an impact on individual parents' decisions concerning vaccinations.

Conclusions

Parents of children with chronic diseases usually have greater concerns regarding vaccinations compared with the general population. In such a case physicians should be able to explain to the parents that their child is more susceptible to infection. It is important to provide comprehensive information about the diseases that can be prevented by vaccination and about different prevention options. Such a conversation requires time, sufficient level of knowledge and communication skills.

Globally observed activity of anti-vaccine movements, vaccine hesitancy and low vaccine coverage rates may have a negative impact on the health of patients whose delays in the implementation of vaccinations result from objective medical causes. Knowledge about the scale of epidemiological threats and the effects of diseases that can be prevented by vaccination may promote the emergence of pro-health social behaviors.

Popularization of knowledge about the clinical trials assessing safety and efficacy of vaccines is one of the ways of trust-building and active vaccine hesitancy prevention.

There is a need to improve the qualifications of medical staff by highlighting the importance of vaccinations in the syllabuses of all medical faculties, whose graduates may influence patient's and caregiver's individual decisions related to vaccination.

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CASUISTIC PAPER

Seweryn Bartosz [1 (ABCDG)], Natalia Leksa [1,2 (ABCDFG)], Dominika Uberman-Kluz [1,3 (ABCDG)], Artur Szymczak [2(FG)], Marek Biesiadecki [2 (FG)], Sabina Galiniak [2 (DFG)]

Rapidly progressing dementia as a manifestation of the Creutzfeldt-Jakob disease: an analysis of two cases

[1] Department of Neurology, MSWiA Hospital, Rzeszów, Poland
[2] Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[3] Faculty of Pedagogy, University of Rzeszów, Rzeszów, Poland

ABSTRACT

Introduction. Creutzfeldt-Jakob disease (CJD) is a rare and fatal neurodegenerative disease of the central nervous system which is caused by an infectious protein called prion. Multiple forms of CJD have been classified including sporadic (more than 90% cases), familial, iatrogenic and variant type of disease. CJD, especially in its early stages, is a highly challenging illness to diagnose.

Aim. Article aims to present cases of Creutzfeldt-Jackob disease with early symptoms of rapidly progressing dementia at the initial stage of CJD.

Description of the cases. This paper describes two cases of patients with suspected CJD with a history of rapidly progressive dementia admitted to the Department of Neurology, MSWiA Hospital in Rzeszów.

Conclusion. Despite the fact that CJD is an incurable illness and there is no cure guaranteeing recovery, it is important to make the right diagnosis. Assay of 14-3-3 protein in cerebrospinal fluid is a sensitive and specific marker which is helpful in the diagnosis of CJD. The only relevant method of correctly confirming a diagnosis of this disease is by performing a brain biopsy.

Keywords. 14-3-3 protein, brain biopsy, cerebrospinal fluid, Creutzfeldt-Jakob disease, EEG, prion

Introduction

Creutzfeldt-Jakob disease (CJD) is a peculiar disease that can not only have a genetic background or occur sporadically, but it can also have an infectious character.[1]

Creutzfeldt-Jakob disease was described for the first time in 1920 by H.G. Creutzfeldt from Breslau (Wroclaw) University.[2] It is the most common of the prion diseases, with a prevalence equal to 1-1.5 per 1,000,000 inhabitants.[1] However, CJD diagnosis is the first diagnosis in only 35% of the patients who probably have the disease.[3]

CJD is caused by the transformation of the normal cellular prion protein (PrP), which is observed on the cell membranes in human and animal subjects, to ab-

Corresponding author: Natalia Leksa, e-mail: nleksa@ur.edu.pl

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

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normal. It has been discovered that the PrP^{c} prion (as *cellular*) is an infectious factor, which undergoes conversion into a proteinase-resistant PrP^{d} (as *disease*) or PrP^{Sc} (as *scrapie*) protein. The PrP protein codes the PRNP gene on the short arm of the 20th chromosome.[4] The disease phenotype as well as the susceptibility of its sporadic form is influenced mostly by the 129 codon of the PRNP gene coding methionine or valine. A vast majority of individuals suffering from the iatrogenic and sporadic forms are considered homozygotes in the 129 codon while sporadic heterozygotes suffer from the disease after a significantly longer latent period. There are also reports of the isolation of a proteinase-sensitive PrP protein from CJD patients which may undermine the theory of the prion being the only etiological factor.[5,6] The following subtypes can be distinguished, based on aetiology:[7]

- Spontaneous (sporadic) – *s*CJD
- Familial (genetic) – *f*CJD
- Iatrogenic (induced) – *j*CJD
- Variant – *v*CJD

The most common form of this disease in Poland, as well as worldwide, is *s*CJD in approximately 90% of all cases.[1,7] The prevalence is equal to 0.9 per million inhabitants. There have been no *v*CJD cases reported in Poland so far.[1] The characteristic clinical image of the disease consists of rapidly progressing dementia, myocloni, visual impairment, cerebellar disturbances, pyramidal or extrapyramidal symptoms, akinetic mutism, characteristic EEG records and 14-3-3 protein presence in the cerebrospinal fluid, when the disease duration is less than 2 years.[3,9] Nonspecific prodromal symptoms occur in 10-30% of patients and include weakness, sleep disorders (insomnia or hypersomnia), eating disorders, or depression.[3,10,11] In the course of the disease, extrapyramidal and cerebellar symptoms dominate over pyramidal. All of the mentioned symptoms rarely occur simultaneously and their order of appearance as well as co-occurrence is largely dependent on the disease subtype. The disease always results in death. It is known that 90% of patients die within a year from the appearance of first symptoms.[12] Due to a heterogeneous clinical image, the following clinical criteria of CJD diagnosis have been developed:[13]

I – rapidly progressing dementia

II - myocloni
- visual impairment
- pyramidal/extrapyramidal symptoms
- akinetic mutism

III – characteristic EEG record (periodically occurrence of sharp and slow waves)

IV – in the magnetic resonance image: a hyperintensive signal in the caudate nucleus and the putamen or in at least 2 areas of the cerebral cortex (temporal, parietal or occipital) in the diffusion-weighted imaging (DWI) or in the fluid-attenuated inversion recovery (FLAIR) – the examination should be conducted using a high field device (min. 1.5 T).

Possible CJD – I + two group II symptoms + duration time lower than years.

Probable CJD – I + at least two symptoms from group II or III, or I + at least two symptoms from groups II + IV, or possible disease + 14-3-3 protein presence in the cerebrospinal fluid.

Definitive CJD – diagnosis of typical histopathological changes and/or PrP^{Sc} deposits as a result of a neuropathological brain examination (biopsy/autopsy).

Description of the case report

Case I

Patient C.K., a 73-year-old male pensioner, was admitted to the Neurology Department due to increased speech impediments, balance disorders, involuntary limb movements, rapidly progressing dementia and double vision sensation. The initial symptoms, which occurred two months earlier, included balance and cognition disorders (at the time the patient was admitted to the Neurology Department and was diagnosed with mild cognitive disorders and cerebellar ataxia). The symptoms underwent significant intensification between the hospitalizations. Furthermore, the medical history included hypertension and type 2 diabetes. At the time of admission, the patient was conscious and demonstrated psychomotor slowness as well as disartric, slow and quiet speech. The physical examination revealed a slight 'central' paresis of the right facial mimic muscles, slightly increased muscular tension in the right limbs, significant limb and torso ataxia, marked myocloni in the upper and lower limbs, medium frequency resting tremor of the upper limbs intensifying with a slight (including vocal) stimulus, right-sided Babinski sign and ataxic, broad-based gait in a bent-back position (impossible independently). Firstly, an MRI with contrast was performed which revealed atrophy of the cerebral cortex and cerebellum and hyperintensive (in T_2-weighted images) paracentral sections of the temporal lobes which did not clarify the reason for the neurological state. Dementia features with distinctive bradyphrenia were observed during the psychological examination. Correct records were obtained in the EEG examination conducted twice. In the differential diagnostics, the prion disease (cerebrospinal fluid was collected to determine the presence of the 14-3-3 protein – positive result) as well as the parkinsonism plus syndrome (lack of clinical improvement after the withdrawal of levodopa and clonazepam) were taken into account. During the hospitalization, the patient's state deteriorated rapidly with the occurrence of sleepiness, verbal contact cessation, the occurrence of global anxiety, intensification of limb and facial myocloni, the appearance of choreic move-

ments, limitation of eyeball side and upward mobility, the appearance of release symptoms, significant increase of muscular tension (pyramidal stiffness) and the appearance of a both-sided Babinski sign. Furthermore, obstructions of the digestive system, fever and pneumonia appeared. The patient died on the 15th day of the hospitalization. A brain autopsy examination was conducted which confirmed the diagnosis of a CJD-type spongiform encephalopathy.

Case II

Patient M.R., a 78-year-old female resident of a Nursing Home, was admitted to the Neurology Department due to a paresis of the right limbs persisting for a few days. According to the available documentation, in the past the patient went through an ischemic stroke of the right hemisphere which resulted in a left-sided hemiparesis; furthermore, the intelligence collected from the Nursing Home staff mentions a rapidly progressing dementia within the period of two months. At the time of admission, the patient demonstrated psychomotor slowness and maintained basic verbal contact (basic information on oneself); the physical examination revealed the following deviations from the normal state: approximately 3 cm neck stiffness, shallowed left nasolabial fold, spastic tetraparesis (more intense on the right side), right ankle clonus and left-sided Babinski sign. The conducted examinations (CT and MRI of the head) revealed a cortical-subcortical brain atrophy as well as symmetrically hyperintensive periventricular changes (in the T_2-weighted MRI images) in the form of degenerative lesions of white matter with overlaying ischemic, angiogenic changes. No fresh pathological foci were found. During hospitalization, several episodes of simple motor seizures of the right limbs were observed. The patient's state deteriorated rapidly with the occurrence of fluctuating consciousness and the myocloni of the lower right limb.

A spinal puncture was conducted obtaining a normal pressure cerebrospinal fluid which was of correct composition. The twice-conducted EEG record revealed periodic, occurring every 1s, discharges of sharp-slow-wave complexes and slow waves. Cerebrospinal fluid was collected again and was positively tested for the presence of the 14-3-3 protein. The patient was discharged to the Nursing Home with severe consciousness disorders, weak reactions to pain stimuli, spastic tetraparesis, right ankle clonus and a lack of plantar reflexes. Further fate of the patient is not known. The clinical characteristics of the patients are given in Table 1.

Discussion

Based on the clinical symptoms, outcomes from EEG and MRI as well as a positive result in 14-3-3 analysis, the abovementioned cases of patients were diagnosed with CJD. A very rapidly progressive dementia is a dominant symptom of CJD and it reveals initial symptoms appeared two months before and very distinctive ones occur in the last week prior to admission.[14] Similarly as in the CJD case described by Yegya-Raman et al. as well as Hamlin et al. cerebrospinal fluid analysis was positive for protein 14-3-3.[9,15-17] Other markers of CJD in the cerebrospinal fluid also include S-100 protein, neuron-specific enolase, or tau. Analysis of tau is considered as a more sensitive and specificity marker in the diagnosis of CJD than 14-3-3 protein. However, combination of determination of tau and 14-3-3 is used as the most efficient marker for the diagnosis of CJD.[17] Recently, there have been reports of a supravital definitive CJD diagnosis on the basis of the amplification of the pathological prion protein from the cerebrospinal fluid with 80% sensitivity and 100% specificity.[6] However, study by Peckeu et al. based on autopsy of 1572 patients with CJD, showed that induction of analysis of 14-3-3 protein caused an increase in the diagnostic sensitivity to 82% and a decrease in the specificity to 75%.[18]

Myoclonus is another important and cardinal symptom for the diagnosis of CJD which occurs in 80-100% of the patients, particularly in the advanced stages of the disease.[19] One of presented cases featured an incorrect periodic EEG record. Literature data shows that EEG criteria

Table 1. Patient characteristics

Age	Sex	Clinical symptoms	Initial diagnosis	MRI	EEG	14-3-3 protein	Biopsy	Times of symptoms
73	M	Sudden significant deterioration of the overall state, speech disorders, involuntary limb movements, rapidly progressing dementia, double vision sensation	Neuro-infection	Cerebral and cerebellar atrophy, hyperintensive paracentral sections of the temporal lobes in T2-weighted images	Recording within norm limits	Present	Disease confirmed	21days
78	F	Paresis of the right limbs	Vascular incident	Supratentorial hyperintensive periventricular changes (in the T_2-weighted MRI images) in the form of degenerative lesions with overlaying angiogenic changes	Discharges of sharp-slow-wave complexes and slow waves occurring periodically approximately every 1 second	Present	Not conducted	16 days

are positive in above 60% of the CJD patients.[20] Magnetic resonance imaging is a helpful tool in the clinical diagnosis of CJD with solid sensitivity and reliable specificity.[21] It is worth mentioning that the MRI examinations did not reveal the characteristic changes in first case. Lack of a description of characteristic changes in the MRI image in CJD may also result from the fact that the such hyperintensive changes in the basal ganglia and the thalamus can be observed in the T_2-weighted images only in certain types (type 1 MM1/MV1, type 2 VV2, type 3 MV2; the rest do not show typical changes). It is also worth mentioning that the image unusualness, the disease rarity and close coordination with the radiologist is of significant importance.[22,23] In the second case the image is the closest to the characteristic change (cortical damage and damage in the proximity of subcortical nuclei) similar to the case described by Kojima.[24,25] Our patients had no history of the disease in family. Evaluation of brain tissue obtained by biopsy or autopsy by neuropathologists is the only way to confirm a diagnosis of CJD.[26] In case of disease, histopathological evidence of neuronal, glial and spongiform damages are revealed.[21]

It was possible to confirm the disease by brain autopsy in only one case; the second patient was discharged from the hospital and it was not possible to convince the family to consent to an autopsy following the patient's death, should it occur outside the facility. In the first case, it is possible to diagnose a probable CJD. The most serious doubts arise in relation to the second case, however, despite the lack of the most typical symptoms and according to the applicable Edinburgh criteria, it is possible to make a probable (rapidly progressing dementia + pyramidal/extrapyramidal symptoms and akinetic mutism + MRI image) or at least possible (without MRI, but the symptoms last less than two years) diagnosis. The greatest problem in making a definitive diagnosis is the lack of the families consent for the autopsy following a discharge from the hospital. Currently, there is no effective method of disease treatment or slowing, therefore the observation and diagnostics should be conducted thoroughly, as a probable diagnosis inclines the inevitability of treatment failure. Furthermore, relaying such information to the family needs to be supported by substantial evidence.

Summarizing, Creutzfeldt-Jakob disease is generally a diagnostic challenge for physicians because symptoms are similar to rapidly progressing dementias, so physicians should have a comprehensive understanding of CJD. It is important to carry out more detailed and sensitive tests to support an early clinical diagnosis, even before the onset of specific clinical manifestations.

Conclusion

In case of a rapidly progressing dementia with apparent neurological symptoms, CJD should be considered. Obviously, the clinical symptoms covered in the diagnosis criteria may appear in various fully-developed neurodegenerative diseases, however a very rapid progression suggests CJD (with the rare exceptions of slow-progression cases e.g. heterozygotes of the PRNP gene 129 codon).

The most characteristic additional examination results rarely occur simultaneously, therefore their lack should not constitute a decision to cease the CJD-oriented observations such as the characteristic periodic EEG record occurs mainly in MM1 homozygotes and MV1 heterozygotes in the PRNP gene 129 codon (type 1 *s*CJD) which constitute approximately 40% of CJD cases.

A clearly lower recognizability in Poland, compared to Europe, may result from the fact that the disease is not diagnosed.

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CASUISTIC PAPER

Filip Wołoszyn [1(ABCFG)], Anna Czmil [2(CDE)]

Upper limb analysis measured by inertial measurement unit tool: a case report

[1] Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[2] Faculty of Electrical and Computer Engineering, Rzeszów University of Technology, Rzeszów, Poland

ABSTRACT

Introduction. This article reports differences in accurate and inaccurate forehand and backhand strokes in tennis. The tests were carried out on a professional tennis player. The duration of a stroke, the heights of the individual segments of the right upper limb and differences in the heights of the segments at the beginning and at the end of every phase of stroke were examined.

Aim. The major aim of the work was to expose upper limb disparity in stokes. **Description of the case report.** The research tool was inertial motion sensors (IMS) based on an accelerometer, a gyroscope and a magnetometer. A professional tennis player was examined using the individual case method and kinematic analysis.

Results. The analysis concerned the average time to perform forehand and backhand strokes during all phases of the stroke, i.e. preparation, acceleration and follow-through phases. The average heights of the individual upper limb segments during a stroke were also taken into account. The results of the study are meant to show how the movement of the upper limb affects the accuracy and velocity of a stroke.

Conclusion. The movements of individual segments in some accurate strokes were similar to those in inaccurate strokes.

Keywords. biomechanics, IMU, tennis, timing, upper limb

Introduction

It is generally accepted that sport results are affected by variables such as motor preparation, psychological preparation, physical development of the body, biomechanical conditions, tactics and also nutrition, genetics, general health, well-being and socio-cultural factors.[1] The main issue explored in this article is biomechanics in tennis, and, more specifically, the kinematic analysis of forehands and backhands.

Biomechanics is applied in many scientific fields, such as physiotherapy, physical education and sports. Biomechanical research can be successfully applied in various sports disciplines. To learn more about movement and its structure, kinematic studies are conducted. They help athletes achieve the best results at the highest level. Many modern methods have been recently used in tennis, such as optoelectronics and systems for three-dimensional kinematic motion analysis.[2] An instructor or

Corresponding author: Filip Wołoszyn, e-mail: filip.woloszyn@gmail.com

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trainer gets natural feedback during training from the sensory system. Recently, feedback has been provided by technical equipment and sports equipment.[3]

Optical motion capture systems are today's trend in the assessment of human movement. These systems are expensive but they provide reliable analysis and reduce the accumulation of laboratory data. Devices such as inertial measurement units (IMU), or IMS, have been invented as an alternative research tool for studying motion kinematics. The advantage of these innovative devices is mobility, which allows testing outside the laboratory. IMUs available on the market have various applications. Their main purpose is to identify motion disorders.[4]

The first step in the analysis of motion in sport when using the feedback system is to obtain a signal. The motion capture system (MCS) technique is important in obtaining feedback in research. Most of these systems are based on different optical systems and inertial sensors. Athletes' movements are captured by measuring various physical quantities, such as acceleration, velocity, position, angular velocity, rotation, angle, force, power and energy. Optical MCSs generally show the spatial position of markers. Inertial sensors based on the MCS show acceleration through an accelerometer, angular velocity through a gyroscope and orientation in space through a magnetometer. Markers are most commonly placed on the athlete's body and should not block or absorb his movements.[2] The quantity of forces through the elbow during tennis stroke can make enormous elongation and valgus overload in players. Tennis has been represented as a power game cause of the explosive physical action of the players and very high ball rapidity.[5]

A tennis game consists of many shots and movements that are performed to accurately hit the ball into the court. Forehands and backhands are the most frequent strokes used by tennis players during a game.[6]

In professional tennis, in addition to serves, forehands and backhands are responsible for the largest number of points gained. In the 2007 US Open, forehands performed by Roger Federer and Novak Djoković, two of the best tennis players in history, accounted for 29.2% and 31.2%, respectively, of all strokes made. For comparison, their backhands accounted for 33.4% and 34.2%, respectively, of all shots performed.[7]

The aim of the study is to find and describe the correct motion patterns based on the basics of biomechanics of solid bodies and, more specifically, biomechanics in tennis. The study focuses primarily on the impact of variables, such as the duration of a stroke and the heights of individual upper limb segments, during a stroke on its accuracy and the velocity of the ball. The study primarily involved forehands and backhands, which largely determine success in scoring points and the game itself. Forehands and backhands consist of many phases and in-depth analysis of every phase allows for broader understanding than if strokes were explored as a whole.

Description of the case report

The study involved a professional tennis player. As a junior, he was very successful at both national and international tournaments. He reached doubles and singles semi-finals and finals at inter-university level. He was a multiple winner of the Polish Junior Championships and participated in International Tennis Federation (ITF) tournaments. The subject took part in the study voluntarily and was informed in detail about the purpose of the tests. The tests took place during one training session.

In this study, the individual case method was used, which was helpful in evaluating the results obtained by the tennis player. The individual case method helped analyse the tennis player being examined. "Axis Neuron" software was the research tool. The device consists of a system of modules and are based on IMU. The apparatus consists of triaxial gyroscopes, triaxial accelerometers and triaxial magnetometers. Accurate and real motion with minimal delay was obtained based on the dynamics of the human body and algorithms.

Analysis of the forehand. Place: Gym with lines marking out the tennis court. Equipment: MCS, IMU, tennis racket, radar. The conduct of the test: Initially, the device was calibrated to produce reliable results. The subject took the basic tennis position around 1 m from the baseline on the right hand corner of the tennis court. The tennis player performed a forehand stroke on the spot, after dropping the tennis ball perpendicular to the ground. The ball dropped by gravitational force from a height of 2 m to the forehand side of the subject. The tennis player had to strike the ball after it bounced off the ground into a designated area on the other side of the net, which was positioned diagonally near the baseline at the corner. This area was a square of 4 m^2. After the stroke, the subject returned to the starting position. The tennis player was asked to perform a total of 36 forehand strokes with the entire system being recalibrated after each series of six strokes. Evaluation: The accuracy of the forehand stroke and the velocity of the ball were evaluated. All registration data and export data were sent directly to the recording equipment via motion sensors.

Analysis of the backhand. Place: Gym with lines marking out the tennis court. Equipment: MCS, IMU, tennis racket, radar. The conduct of the test: Similar to the forehand strokes, the device was calibrated to produce reliable results. The subject took the basic tennis position around 1 m from the baseline of the court on the left hand corner of the tennis court. The tennis player performed a backhand stroke on the spot, after dropping the tennis ball perpendicular to the ground. The

ball dropped by gravitational force from a height of 2 m to the backhand side of the subject. The tennis player had to strike the ball after it bounced off the ground into a designated area on the other side of the net, which was positioned diagonally near the baseline at the corner. This area was a square of 4 m^2. After the stroke, the subject returned to the starting position. The tennis player was asked to perform a total of 36 backhand strokes with the entire system being recalibrated after each series of six strokes. Evaluation: The accuracy of the backhand stroke and the velocity of the ball were evaluated. All registration data and export data were sent directly to the recording equipment via motion sensors.

Fig. 1. Photos presenting tennis player in (IMU) "outfit"
Source: Own elaboration

Results

The analysis concerned the average time to perform forehand and backhand strokes during all phases of the stroke, i.e. preparation, acceleration and follow-through phases. The average heights of the individual upper limb segments during a stroke were also taken into account. The results of the study are meant to show how the movement of the upper limb affects the accuracy and velocity of a stroke. They also demonstrate when the strings of the tennis racket hit the ball. Differences in the height of every limb segment examined correspond to the initial phase and the final phase of each stroke section described, e.g. the beginning and the end of the follow-through phase. The analysis involved three accurate forehand and three accurate backhand strokes, as well as three inaccurate forehand and three inaccurate backhand strokes.

The first stroke analysed (Figure 2) was the forehand, which was considered an accurate stroke. The velocity of the ball during this test was 105 km/h. The time of this stroke from the preparation phase to the follow-through phase was 1.05 s. The preparation phase, i.e. abduction of the racket hand, lasted 0.43 s. The duration of the acceleration phase was approximately 0.48 seconds. The strings of the racket hit the ball at the end of the acceleration phase, at 0.9 s of the stroke. The follow-through phase lasted 0.15 s. The heights of the hand

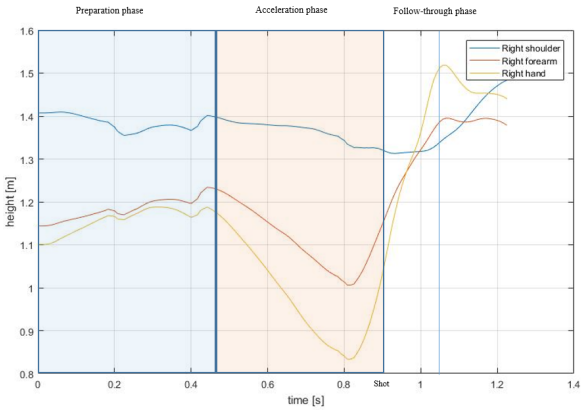

Fig. 2. Accurate forehand stroke

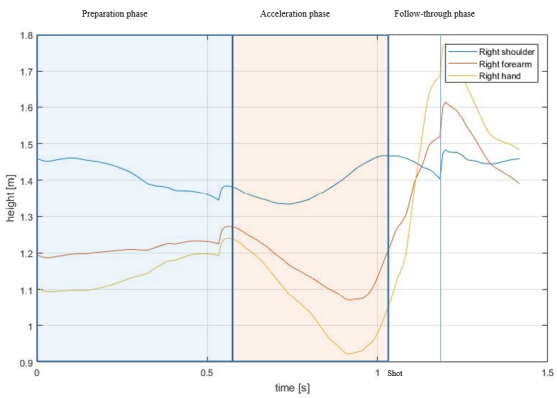

Fig. 3. Inaccurate forehand stroke

in the preparation phase oscillated from 1.1 m to 1.18 m. The heights of the forearm in the preparation phase were close to the heights of the hand and ranged from 1.15 m to 1.2 m. In the preparation phase, the shoulder was at around 1.4 m without a noticeable change in height and dropped slightly at the end of the phase. Significant height amplitude was observed during the acceleration phase in each segment of the upper limb examined, with the exception of the shoulder. The level at which the shoulder was located did not change significantly, decreasing from approximately 1.4 m to 1.3 m. The height of the forearm decreased from 1.22 m to 1.05 m and rose to around 1.15 m at the moment when the ball touched the strings of the racket. In the follow-through phase, the height of the shoulder increased to about 1.3 m and 1.4 m, while the highest amplitude was recorded in the heights of the forearm and the hand: the height of the forearm increased to 1.4 m and the height of the hand rose to 1.52 m. It was observed that the time of the entire second forehand stroke was 0.98 s, i.e. 0.7 s shorter than that of the first one. It was also discovered that the preparation and acceleration phases in the second forehand were significantly shorter, in contrast to the follow-through phase, which lasted longer. The moment of impact occurred earlier, i.e. at 0.79 s of the entire stroke. Differences in heights

in the preparation phase were not clear, but the difference in the heights of the hand in the acceleration phase amounted to 0.09 m. In the follow-through phase, a noticeable difference was noted in the heights of the shoulder amounting to 0.09 m. Attention should also be paid to the fact that the hand moved higher than in the first stroke. At the lowest point, it dropped to 0.94 m, while in the first stoke it reached 0.84 m. In the third forehand stroke, the difference in the heights of the hand between the beginning and the end of the preparation phase was significantly greater amounting to 0.22 m. Interestingly, the forearm was higher than the hand in the preparation phase. The next three forehands tested were considered as missed. The differences observed range from a few to several centimetres and from hundredths to tenths of a second. The velocities of missed balls hit by the tennis player were on average comparable to those of accurate strokes. The duration of a missed stroke was on average

Table 1. Data of accurate forehand stroke

	F_1	F_2	F_3	$\overline{X}$
Accuracy	in	in	in	**100%**
Ball speed (km/h)	105	115	121	**113.67**
Stroke duration (s)	1.05	0.98	1.00	**1.01**
Length of preparation phase (s)	0.43	0.39	0.35	**0.39**
Length of acceleration phase (s)	0.48	0.40	0.52	**0.47**
Length of follow-through phase (s)	0.15	0.19	0.13	**0.16**
Moment of impact (s)	0.90	0.79	0.87	**0.85**
Height disparity of hand in preparation phase (m)*	0.08	0.06	0.22	**0.12**
Height disparity of forearm in preparation phase (m)*	0.08	0.07	0.10	**0.08**
Height disparity of shoulder in preparation phase (m)*	0.01	0.02	0.06	**0.03**
Height disparity of hand in acceleration phase (m)*	0.23	0.14	0.12	**0.16**
Height disparity of forearm in acceleration phase (m)*	0.08	0.07	0.08	**0.08**
Height disparity of shoulder in acceleration phase (m)*	0.09	0.09	0.11	**0.10**
Height disparity of hand in follow-through phase (m)*	0.52	0.54	0.56	**0.54**
Height disparity of forearm in follow-through phase (m)*	0.27	0.27	0.25	**0.26**
Height disparity of shoulder in follow-through phase (m)*	0.03	0.12	0.07	**0.07**

* Difference in the heights of the segment between the beginning and the end of the phase. The result is given in absolute value.

Table 2. Data of inaccurate forehand stroke

	F_4	F_5	F_6	$\overline{X}$
Accuracy	out	out	out	**100%**
Ball speed (km/h)	117	113	120	**116.67**
Stroke duration (s)	0.94	1.05	1.20	**1.06**
Length of preparation phase (s)	0.39	0.50	0.60	**0.50**
Length of acceleration phase (s)	0.43	0.32	0.42	**0.39**
Length of follow-through phase (s)	0.13	0.23	0.18	**0.18**
Moment of impact (s)	0.81	0.82	1.02	**0.88**
Height disparity of hand in preparation phase (m)*	0.21	0.26	0.13	**0.20**
Height disparity of forearm in preparation phase (m)*	0.10	0.19	0.06	**0.12**
Height disparity of shoulder in preparation phase (m)*	0.09	0.01	0.08	**0.06**
Height disparity of hand in acceleration phase (m)*	0.17	0.26	0.21	**0.21**
Height disparity of forearm in acceleration phase (m)*	0.02	0.06	0.07	**0.05**
Height disparity of shoulder in acceleration phase (m)*	0.03	0.08	0.07	**0.06**
Height disparity of hand in follow-through phase (m)*	0.42	0.75	0.78	**0.65**
Height disparity of forearm in follow-through phase (m)*	0.20	0.42	0.41	**0.34**
Height disparity of shoulder in follow-through phase (m)*	0.00	0.04	0.02	**0.02**

* Difference in the heights of the segment between the beginning and the end of the phase. The result is given in absolute value.

0.05 s longer than that of an inaccurate stroke. In the third stroke, the value was 1.2 s while the duration of accurate strokes was at a constant level of around 1 s. The swing in inaccurate strokes was also longer, unlike the acceleration phase. The duration of the stroke in the follow-through phase was on the same level. The estimated moment of impact was similar in accurate and missed strokes. Differences in the heights of the upper limb segments in the preparation phase in inaccurate stokes did not significantly differ from those in accurate strokes. The differences in the heights of the hand, the forearm and the shoulder between the beginning and the end of the acceleration phase were 0.05 m, 0.03 m and 0.04 m respectively. While greater differences in the heights of the hand and the forearm were observed in missed strokes, the differences in the heights of the shoulder in inaccurate strokes were small. In some missed strokes, in addition to differences in the time taken performing a stroke, attention should be paid to differences in the values of individual strokes at impact, differences in the heights of individual segments in the preparation phase, and the heights of the shoulder, the hand and the forearm in the acceleration phase.

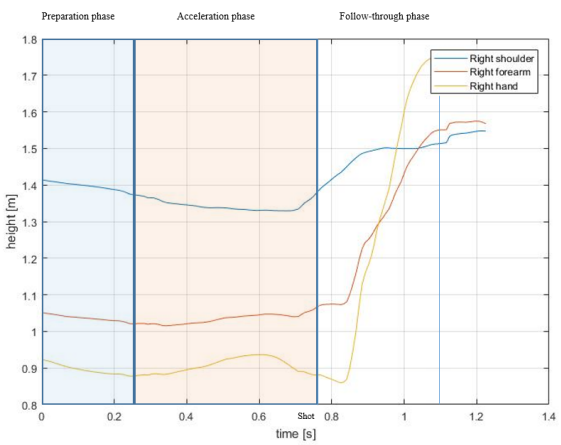

Fig. 4. Accurate backhand strok

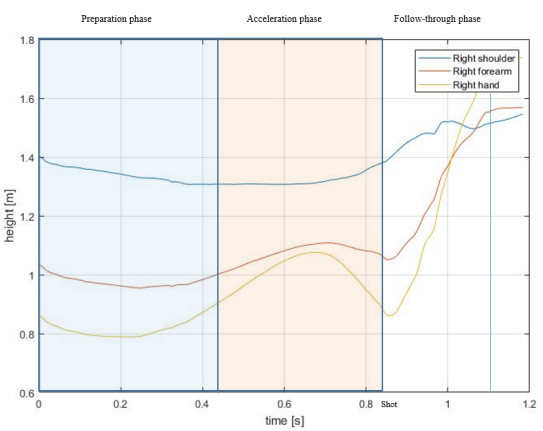

Fig. 5. Inaccurate backhand stroke

Backhands were also divided into three accurate and three inaccurate strokes. The method and technique of the tests used to obtain the results diagnosing backhand strokes was analogous to those used in the forehand stroke test. It is worth mentioning that the tennis player preferred two-handed backhands.

The velocity of the ball after the first backhand stroke was 96 km/h. The stroke was performed in 1.10 seconds. The duration of the backhand was similar to that of the forehand. The moment of impact was estimated at 0.78 s of the entire stroke. The acceleration phase was the longest phase of the stroke. In the first backhand analysed, attention should be paid to the height of the racket trajectory and, consequently, the upper limb elevation because the in-depth analysis of the first stoke did not show particularly large changes in height between the preparation phase and the acceleration phase. It can therefore be said that the swing and acceleration of the tennis racket were horizontal. The heights of the forearm and the hand only changed significantly in the follow-through phase. The average velocities of the balls after the strokes were 101 km/h in the second case and 109 km/h in the third case. With each attempt, the player increased the accuracy of strokes. The average velocity of all 36 backhand balls was more than 104 km/h, and the average speed of 36 forehand balls was more than 114 km/h. As in the case of the first stroke, in the second accurate stroke, the racket moved practically horizontally. In this attempt, the moment of impact was delayed and the swing lasted longer. Only the height of the shoulder changed significantly. The difference in height measured from the beginning to the end of the phase was 28 cm larger than at the first attempt. Differences in the heights of the shoulder, the forearm and the hand did not change much when considering the initial and the final phases of the third stroke. It was observed that the racket moved along a larger parabola than in the two previous strokes, which apparently did not affect the accuracy of the stroke. There was a noticeable change in the height of the hand in the follow-through phase of the third stroke and there were large differences in the heights of the shoulder in all accurate backhands.

The first backhanded tested was characterised by a similar racket trajectory to the first two accurate backhand strokes. The trajectories of the second and third inaccurate backhands were similar to that of the last accurate backhand stroke. In the second missed backhand, the hand moved higher than the forearm in the acceleration phase. A similar situation occurred with the third forehand. Missed backhands were characterised by a slightly higher velocity of the ball after the stroke, amounting, on average, to more than 2 km/h than in the case of accurate strokes. The average duration of the stroke was practically identical; however, the difference in duration between certain strokes was

0.14 s, i.e. around 11% of the longest stroke. The average difference in the duration of the preparation phase between accurate and inaccurate strokes was 0.06 s. The longest swing in all forehands tested was found to be inaccurate. The difference in the mean lengths of the acceleration phase is not large and amounts to 0.01 s. In all the strokes tested, there are differences in the parabolas of the upper limb motion in the acceleration phase. In the fourth backhand, the preparation and acceleration phases were longer than the follow-through phase. The moment of impact in missed strokes occurred on average 0.05 s later than in accurate strokes. In the fourth backhand, attention should be paid to the result of the follow-through phase and the moment of impact. The longer swing and acceleration phases and the shorter follow-through phase and delayed stroke may correlate with each other. This can be explained as "making up for" bad timing with a shorter follow-through phase.

Discussion

The research on forehand and backhand biomechanics focuses primarily on the areas of medicine and rehabilitation. The areas of research are diverse and refer to many factors making up a tennis game. For example,

Table 3. Data of accurate backhand stroke

	B_1	B_2	B_3	$\overline{X}$
Accuracy	in	in	in	**100%**
Ball speed (km/h)	96	101	109	**102.00**
Stroke duration (s)	1.10	1.24	1.12	**1.15**
Length of preparation phase (s)	0.23	0.40	0.38	**0.34**
Length of acceleration phase (s)	0.53	0.51	0.39	**0.48**
Length of follow-through phase (s)	0.32	0.33	0.35	**0.33**
Moment of impact (s)	0.78	0.91	0.77	**0.82**
Height disparity of hand in preparation phase (m)*	0.05	0.02	0.13	**0.07**
Height disparity of forearm in preparation phase (m)*	0.04	0.03	0.01	**0.03**
Height disparity of shoulder in preparation phase (m)*	0.04	0.05	0.14	**0.08**
Height disparity of hand in acceleration phase (m)*	0.07	0.19	0	**0.09**
Height disparity of forearm in acceleration phase (m)*	0.02	0.06	0.02	**0.03**
Height disparity of shoulder in acceleration phase (m)*	0.11	0.02	0.03	**0.05**
Height disparity of hand in follow-through phase (m)*	0.85	0.72	0.59	**0.72**
Height disparity of forearm in follow-through phase (m)*	0.47	0.44	0.45	**0.45**
Height disparity of shoulder in follow-through phase (m)*	0.02	0.34	0.19	**0.18**

* Difference in the heights of the segment between the beginning and the end of the phase. The result is given in absolute value.

Table 4. Data of inaccurate backhand stroke

	B_4	B_5	B_6	$\overline{X}$
Accuracy	out	out	out	**100%**
Ball speed (km/h)	94	110	109	**104.33**
Stroke duration (s)	1.18	1.14	1.11	**1.14**
Length of preparation phase (s)	0.44	0.34	0.43	**0.40**
Length of acceleration phase (s)	0.56	0.45	0.39	**0.47**
Length of follow-through phase (s)	0.18	0.35	0.29	**0.27**
Moment of impact (s)	1.00	0.79	0.82	**0.87**
Height disparity of hand in preparation phase (m)*	0.10	0.06	0.07	**0.08**
Height disparity of forearm in preparation phase (m)*	0.06	0.04	0	**0.03**
Height disparity of shoulder in preparation phase (m)*	0.05	0.09	0.10	**0.08**
Height disparity of hand in acceleration phase (m)*	0.15	0.20	0.10	**0.15**
Height disparity of forearm in acceleration phase (m)*	0.17	0.13	0.08	**0.13**
Height disparity of shoulder in acceleration phase (m)*	0.09	0.03	0.02	**0.05**
Height disparity of hand in follow-through phase (m)*	0.72	0.53	0.79	**0.68**
Height disparity of forearm in follow-through phase (m)*	0.31	0.31	0.46	**0.36**
Height disparity of shoulder in follow-through phase (m)*	0.07	0.06	0.12	**0.08**

* Difference in the heights of the segment between the beginning and the end of the phase. The result is given in absolute value.

the study of reactions to the change of friction during a game played on a clay surface has shown that the free surface has a significant impact on reducing the risk of injury by increasing the moment of sliding.[8] The issues of diagnosis, management and treatment of injuries to which tennis players are exposed were studied using the example of arm pain during its rotation.[9] Moreover, kinematic analysis of the shoulder joint was conducted as a non-invasive method of studying the kinematic chain of stroke and arm instability.[10] For example in table tennis investigations about forehand are concerned in upper limb kinematics, upper limb kinetics and lower limb kinematics analysis.[11-13] The study concerning the development of methodology to determine the relationship between the size of the grip and the kinematic share of angular velocity is an example of research that focuses on tennis equipment and its impact on the player's performance.[14] Biomechanics in tennis also involved a kinematic comparison of successful and unsuccessful tennis serves across the elite development pathway, where it has been proven that there are no clear differences in body kinematics during serves.[15]

Conclusion

Correlations determined and results obtained through the in-depth analysis lead to the conclusion that the velocity of the ball after forehand and backhand strokes does not affect the accuracy of the stroke. It can be assumed that the ball flying on average 2 km/h faster did not reach the designated place. Considering that the majority of strokes were over 100 km/h, this variable does not affect the achievement of the intended goal. It was noticed that the average duration of accurate and inaccurate strokes was similar, but with visible differences in the duration of individual phases. This can be described as the tennis player's ability to match the duration of the swing, the acceleration phase and the moment of impact to achieve a good result. Height modulation helped the subject perform correct and accurate forehand and backhand strokes. The movements of individual segments in some accurate strokes were similar to those in inaccurate strokes. After comparing accurate and inaccurate strokes, conclusions regarding the movement of the upper limb clearly show that differences in the movements of the hand, the forearm and the shoulder are dependent on the tennis player himself, taking into account the automation of his movements. Most of the analysed strokes do not differ significantly. Some details concerning height and length during the research process were optimised by the subject. If he was an amateur tennis player, the results would be completely different and would show differences in movements of the upper limb during each stroke.

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CASUISTIC PAPER

Filip Wołoszyn (ABCDEFG)

Cardiopulmonary exercise test performed on a football player: a case report

Faculty of Medicine, University of Rzeszów, Rzeszów, Poland

ABSTRACT

Introduction. Cardiopulmonary Exercise Test (CPET) can precisely determine aerobic capacity, conjugate and independent functions of the pulmonary cardiovascular, and skeletal muscle systems.

Aim. To describe CPET feedback from a high stamina professional football player.

Description of the case. The test took place in course of one CPET session. The method of an individual case study was used in this research. The participant was a local team football player. According to the coaches' opinion, this player had the best ability to perform long distance work. The test was performed using a cycle ergometer. Cardiopulmonary Exercise Test was performed with a cycle ergometer RAMP test.

Conclusion. The player's capacity is at a level that allows us to outline his results as a unique case.

Keywords. capacity, circulatory system, CPET, effort, endurance, football, RAMP, respiratory system

Introduction

The Cardiopulmonary Exercise Test (CPET) is a non-invasive and very safe process engaging an estimation of the respiratory and cardiovascular system during exercise to set personal exercise performance and functional capacity.[1] In the last years, CPET has changed from the area of the surveys in laboratories and sports medicine to the direction of clinical practice.[2] By measuring a wide range of different variables, CPET can precisely determine aerobic capacity, conjugate and independent functions of the pulmonary cardiovascular and skeletal muscle systems.[3] The test provides the researcher an indirect survey of cardiopulmonary physiology and fundamental metabolic base in healthy and unhealthy populations.[4] Respirometry is allowed continuously in physical effort and in rest to register oxygen uptake, carbon dioxide production and quantity of minute ventilation.[5] The measurement of gas exchange is carried out breath by breath and the measurement results are almost simultaneously displayed on the computer screen, by fast processing and correction of data, taking into account the delay, which is the transfer of data from the exhalation to the measurement.[6] The respiratory system has a particular value among body's functioning system. A significant and prominent role of this system is to provide the requested energy for organs and different tissues that are affected seriously in short and long term exercises.[7] CPET is a ramped survey during which

Corresponding author: Filip Wołoszyn, e-mail: filip.woloszyn@gmail.com

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

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the blood pressure, electrocardiography and saturations of oxygen are measured. Respiratory variables, including oxygen uptake and carbon dioxide excretion are also quantified. An amount of variables can be derived taking into consideration the oxygen uptake peak, which depends on effort, and oxygen uptake at the anaerobic threshold, which is not related to motivation or stress.[8] Maximum oxygen peak consumption and oxygen consumption at anaerobic threshold have been reported to be good predictors of physical fitness, physical effort and death rate.[9] Oxygen uptake in CPET test is measured in effort with growing load. Increasing of physical effort intensity, through crossing the lactate threshold, provides to reaching maximal oxygen uptake. Maximal oxygen uptake (VO_2max) parameter is the most popular indicator of physical fitness in the healthy population. In this concept we understand maximal oxygen amount used by an organism in one minute.[10] Effort is usually put forth on a treadmill or upright-bicycle. To emphasize the importance of this test, it should be assumptive that direct measurement uptake is the most efficient and appoint measurement method of physical effort assessment.[11]

This review presents the data of a CPET test of a professional football player. Among a group of participants, this particular player is characterized by a huge maximal oxygen uptake compared both to the other players and to the rest of the population.

Description of the cases

The survey was conducted at the University of Rzeszów. The tests were performed in the Laboratory of Cardiopulmonary Research. The method used in this research was an individual case study. The participant was a local team football player. The player, in opinion of coaches, had the best ability to perform long distance work. The subject took part in the study voluntarily and was informed in detail about the aim of the test.

The test took place in the course of one CPET session. Cardiopulmonary Exercise Test was performed using a cycle ergometer RAMP test Wasserman protocol 1. The test was performed using a cycle ergometer in cooperation with a "Metamax 3B" spiroergometry device and "Metasoft Studio" software. For the functioning of the spiroergometry device, other devices like gas sample line, transmitter belt and elastic strap, or face mask were used. First of all, the CPET device was calibrated in order to exhibit authoritative results. Cardiopulmonary Exercise Test equipment was supported under manufacturer conservancy agreements in keeping with manufacturer references.

The player cycled for 6 minutes with no resistance at a rate of 60 rpm. After this time, resistance was increased linearly at 25 W min^{-1}. The participant was also obligated to keep a minimum rate of 60 rpm during the total time of the test. After maximum effort, the patient performed a regeneration phase with no resistance, pedalling in a 60 rpm load. The regeneration phase and CPET was ended when approximate initial indicators reached their values. Standards used to set the maximal effort that was reached were: VO_2 (ml/kg/min) >46, VO_2 (l/min)/% > 3.51, HR max/% > 182, VO_2/HR > 19, VAT (l/min)/% > 80%, BF > 41, VE max (l/min) > 141.7, VE/VCO_2 slope < 30, VE/VCO_2 in AT < 34.[12] The continuous data was assembled using Metamax Toolbox software. The characteristics of the participant are featured in Table 1.

Table 1. Basic biological and medical data of the participant

Height	184 cm
Body weight	76.0 kg
Mask	blue ,small
Race	White / Caucasoid
Fat tissue	7%
BMI	22
Estimation physical fitness	high
BSA	1.98 m^2

Results

Maximally, the participant performed the exercise test to the level of 413W and reached VO_2max equal to 4.96l/min. Data of measured basic variables are presented in tables number 2 and 3. This result is above the expected value of 3.51 l/min (141%). The relative maximal oxygen uptake VO_2/kg amount is 65ml/min/kg. According to AHA Classification, physical fitness is defined as perfect.[13] At maximum effort, the RER ratio of respiratory exchange tallied 33g/h, and the heart rate HR reached 169/min, which corresponds to 94% of the predicted value. The AT anaerobic threshold is at 3.46 l/ min, corresponding to 45ml /min/kg. This represents 99% of the anticipated, and 70% of actually achieved maximum oxygen consumption. Figures 1, 2 and 3 depicted the VT1 and the VT2 threshold and the method of their appointment.

Discussion

Maximum adaptation to endurance training, which is characterized by the world record holders in endurance competitions, requires many years of regular and optimal training. In addition, it is only possible for people with appropriate genetic conditions[10]. Typical maximal values of oxygen uptake for young students ranges from 44 to 55 ml/min^{-1}/kg^{-1}. Values of VO_2max exceeding 60 ml/min/kg were noticed only in master athletes. VO_2 max exceeding 70 ml/min/kg, were investigated in endurance disciplines like (athletics, short and long distance runs, cycling, ski, cross-country skiing).[15] The master athletes' maximal heart rate was stationary (171

Table 2. Data of basic measured variables 1

Variable	Unit	Rest	Futile load	VT1	VT1 % Norm	VT1 % Max
$V'O_2$	l/min	0.61	0.68	3.46	99	70
$V'O_2$/kg	ml/min/kg	8	9	45	99	70
$V'O_2$/HR	ml	9	10	25	127	84
WR	W	0	0	277	87	67
HR	/min	68	67	141	78	83
$V'E/V'O_2$		21.2	21.1	24.5	-	61
$V'E/V'CO_2$		28.6	28.1	26.1	-	71
V'E	l/min	15.2	16.7	89.9	-	43
BF	/min	16	16	36	87	49

Table 3. Data of basic measured variables 2

Variable	VT2	VT2 % Norm	VT2 % Max	$V'O_2$max	$V'O_2$max % Pred	Norm	Maximal absolute values
$V'O_2$	4.52	129	91	4.96	141	3.51	5.17
$V'O_2$/kg	60	129	91	65	141	46	68
$V'O_2$/HR	28	144	96	29	151	19	30
WR	378	118	91	413	129	321	414
HR	162	89	95	169	94	181	171
$V'E/V'O_2$	32.8	-	82	39.9	-	-	50.1
$V'E/V'CO_2$	31.5	-	85	37.0	-	-	37.8
V'E	156.0	-	75	208.2	-	-	212.2
BF	53	127	72	73	176	41	79

+/- 3 beats/min) and their maximal O_2 pulse reduced from 0.32 to 0.30 ml.kg^{-1} beat[16]. When high intensities of exercise describe the training program, taking advantage of heart rate to quantify training loads appears invalid.[16]

Based on surveys in football, systematic recreational football training increases maximum oxygen uptake VO_2max in players, who were previously untrained. Many surveys have depicted 7–15% increases in VO_2max after training of duration 12–24 weeks, which is similar to or higher than observed in surveys with cycling and running.[17] Commonly, peak O_2 and AT fall equally.[18] It has been noted that the variables of the power–time connection are related to different markers of cardio-respiratory fitness.[19]

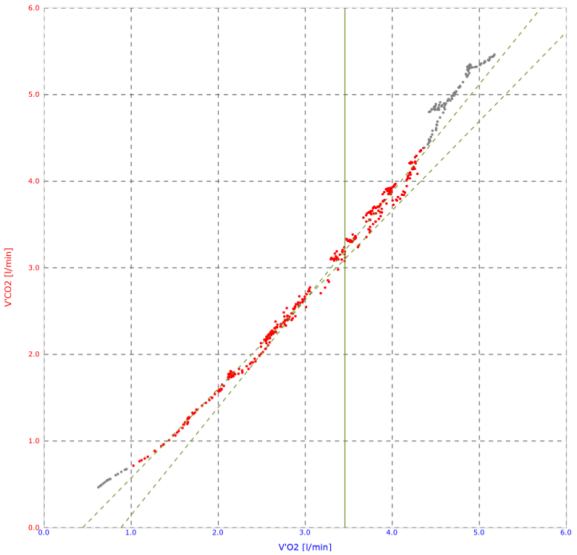

Fig. 1. VT1 appointed on data refined method average apiece sample, with 13 parameters. Range of calculations from 0:07:00 to 0:18:50

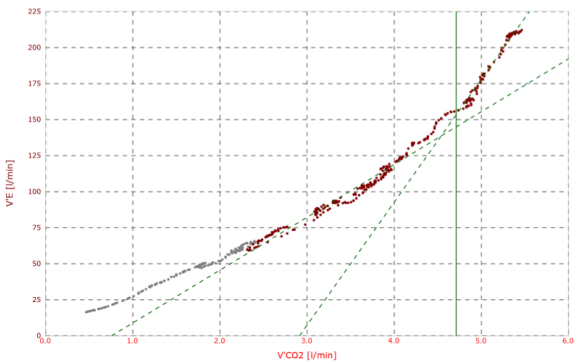

Fig. 2. VT2 appointed on data refined method average apiece sample, with 13 parameters. Range of calculations from 0:13:18 to 0:20:36

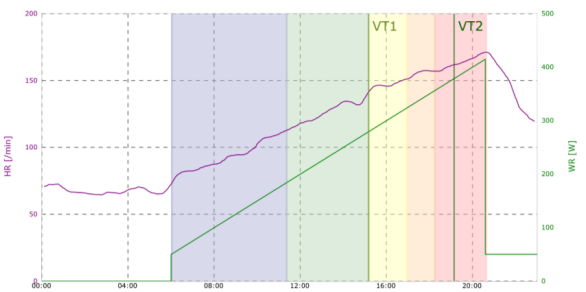

Fig. 3. Zones of exercise intensity. Violet: compensating, green: sustainable, yellow: formative, orange: mix aerobic/anaerobic red: anaerobic (LA). Zones based on manufacturer proposal[14]

The Cardiopulmonary Exercise Test can be used to estimate the rawness of pulmonary hypertension in participants with established illness and provide feedback to therapy. Studies showed that persons with VO_2 peak lower than 10.4 ml kg^{-1} min^{-1} had a worse prognosis.[20]

Conclusion

The tested player achieved $V0_2max$ which ascertained perfect assumptive values. Values of VT1, oxygen pulse, breathing frequency and maximum work performed by the participant can also be acknowledge as perfect. VO_2max, which is the most important indicator of physical fitness, assessing efficiency on level of 65 ml min^{-1}/kg^{-1} is a result, which is reached by top athletes in endurance competitions. Taking into account the testing in laboratory conditions, the perspective of achievement of an even higher VO_2max in natural conditions for the player is presumable.

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