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SECRETARIAT ADDRESS
70 Bukowska Street, room 104
60-812 Poznan, Poland
phone/fax: +48 61 854 72 74
email: jms@ump.edu.pl
www.jms.ump.edu.pl

DISTRIBUTION AND SUBSCRIPTIONS
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60-356 Poznan, Poland
phone/fax: +48 61 854 74 14
email: sprzedazwydawnictw@ump.edu.pl

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WYDAWNICTWO NAUKOWE UNIWERSYTETU MEDYCZNEGO IM. KAROLA MARCINKOWSKIEGO W POZNANIU
60-812 Poznań, ul. Bukowska 70
tel./fax: +48 61 854 71 51

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

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

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ORIGINAL PAPER

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The pharmacokinetics of midazolam and 1-OH-midazolam during oral premedication in paediatric patients

Alicja Bartkowska-Śniatkowska[1], Pawel Wiczling[2], Magdalena Juzwa-Sobieraj[1], Ewelina Kałużna[3], Bogna Świątek-Kościelna[3], Agnieszka Bienert[4], Agnieszka Borsuk[2], Artur Tezyk[5], Jowita Rosada-Kurasinska[1], Danuta Januszkiewicz-Lewandowska[3, 6]

[1] Department of Paediatric Anaesthesiology and Intensive Therapy, Poznan University of Medical Sciences, Poland
[2] Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdansk, Poland
[3] Department of Molecular Pathology, Institute of Human Genetics of the Polish Academy of Sciences, Poznan, Poland
[4] Department of Clinical Pharmacy and Biopharmacy, Poznan University of Medical Sciences, Poland
[5] Department of Forensic Medicine, Poznan University of Medical Sciences, Poland
[6] Department of Oncology, Hematology and Bone Marrow Transplantation, Poznan University of Medical Sciences, Poland

ABSTRACT

Aim. Development of midazolam (MDZ) pharmacokinetic model is pivotal for predicting drug response and determining appropriate dosing in patients who undergo surgical procedures. The aim of this study was to provide population pharmacokinetic analysis describing MDZ and its main metabolite 1-OH-midazolam (1-OH-MDZ) used during oral premedication in surgical paediatric patients. The influence of gender, age, and body weight on MDZ pharmacokinetics was also investigated.

Material and methods. The analyzed data set included 27 patients, aged 1 to 17 years, who received oral midazolam syrup before various surgical procedures. The 1-OH-MDZ concentration was approximated by a proportional relationship to MDZ concentration. Population nonlinear mixed-effect modeling was done using NONMEM 7.2. Non-parametric bootstrap and VPC were conducted to evaluate the adequacy of the model to describe the observations.

Results. Midazolam pharmacokinetic model was developed to describe the time course of MDZ and 1-OH-MDZ concentrations. High inter-individual variability in volume of central compartment (93%) and clearance (60%) of MDZ were observed. The effect of body weight was accounted for by the allometric scaling. Significant differences in MDZ pharmacokinetics due to the age and gender were not found.

Conclusions. The population MDZ pharmacokinetic model was successfully developed for paediatric patients. Age, gender do not explain inter-individual variation in the pharmacokinetics of MDZ. No effect of maturation was detected.

Keywords: midazolam; midazolam pharmacokinetic model; 1-OH-midazolam.

Introduction

Midazolam (MDZ) is a sedative drug, which is also commonly used in premedication of general anaesthesia in diagnostic and surgical procedures. It is a member of benzodiazepines family and exhibits anxiolytic, hypnotic, amnesic, myorelaxant and anti-convulsant properties [1]. Sedative effect results from MDZ interaction with ionotropic gamma-aminobutyric acid receptors

($GABA_A$), which causes the opening of chloride channels and increases the penetration of chloride ions inside the neuron. Anti-anxiety properties are linked to the increasing of the glycine inhibitory neurotransmitter [2]. In critically ill children MDZ is administered intravenously, while in the others orally. MDZ is characterized by a rapid onset and short duration of action as well as a constant efficiency. The highest concentration in plasma is achieved within 30 min [3]. Owing to a first-pass hepatic extraction its bioavailability, after oral administration, is estimated at about 50%. Elimination of MDZ occurs mainly by its hydroxylation by intestinal and hepatic cytochrome P450 A4 (CYP3A4) and A5 (CYPA5) enzymes. In this process two metabolites are formed i.e. 1-hydroxymidazolam (1-OH-MDZ, α-hydroxy MDZ) and 4-OH-MDZ [2]. It was shown that 1-OH-MDZ has sedative properties and may significantly contribute to the effects of MDZ, whereas 4-OH-MDZ is quantitatively unimportant [1]. Finally, both metabolites are conjugated with glucuronide acid and excreted into urine [2]. Studies on pharmacokinetics of MDZ have revealed differences in drug half-life ($t_{1/2}$) and weight-corrected clearance between adults, infants and children that are well accounted from by an allometric principle [4]. Neonates have prolonged $t_{1/2}$ and smaller body weight normalized clearance than adults. Between 1 and 2yr higher body weight-normalized clearance is observed, and then a decline to adulthood [5–7]. Pharmacokinetics studies on different populations are essential to proper dosing of MDZ. It is also important to know factors responsible for inter-individual variations in MDZ pharmacokinetics. There are significant differences in pharmacokinetics of many drugs in children and adults, which justify specific studies on paediatric population [8, 9]. Therefore, the aim of this study was to provide population pharmacokinetic analysis describing MDZ and its main metabolite 1-OH-MDZ concentrations after its oral administration for premedication purposes in children. Moreover, the influence of factors as age, gender and body weight on the population MDZ pharmacokinetics in paediatric patients was investigated.

Material and Methods

Patients

Twenty-seven children scheduled for elective surgical procedures, aged between 1 and 17 years, children of Caucasian ancestry, male (n = 20) and female (n = 7), were enrolled in this study (**Table 1**). Surgical procedures included hypospadia, total or partial thyroidectomy, plastic surgery and tumor removal. The local Ethical Committee of the Poznan University of Medical Sciences approved the study (no. 275/12). Parents of all included patients signed the informed consent on the medical records at the hospital. All experiments were carried out in compliance with the relevant laws and guidelines in accordance with the ethical standards of the Helsinki Declaration.

Patients overall health was assessed as I–II, according to the American Society of Anesthesiologists (ASA) physical status classification system. Exclusion criteria included: physical status ASA III and more, active respiratory infection, metabolic or congenital disorders, sedative or anticonvulsive medication. Twenty-four hours before surgical procedure each patient was managed by anesthesiologist according to the preoperative criteria. All patients were made to be fast overnight, but could drink clear fluids up to 2 hours before the induction of anaesthesia.

Pharmacokinetic study design

Oral MDZ syrup was administered in dose of 0.3 mg kg^{-1} (up to maximum of 15 mg) to the patients as a premedication, from 30 to 45 minutes before surgical procedure. Sedation level was assessed in the operating room using the Richmond Agitation-Sedation Scale (RASS, **Table 2**). General anaesthesia was induced with 2–5% sevoflurane via facemask in children with no intravenous (IV) access, or with propofol intravenously in dose of 2–4 mg kg^{-1}, in those with IV access. During the induction fentanyl in dose 1–2 mcg kg^{-1} was administered to all patients. The airways were maintained by endotracheal intubation or laryngeal mask. Intubation was facilitated by miva-

Table 1. Demographic characterization of patients (n = 27). Results are expressed as median and range for continues and as count for categorical variables

Parameter [unit]	Median [Range]
Male/Female	20 / 7
Age [years]	10 [1.75–17]
Weight [kg]	47 [10.625–90]
MDZ dose [mg]	7.5 [2.5–15]

Table 2. Assessment of sedation in study subjects using the Richmond Agitation-Sedation Scale (RASS), n = 27

RASS score	Term	n, %
2	Agitated	1, 3.7%
1	Restless	5, 18.5%
0	Alert and calm	15, 55.6%
-1	Drowsy	5, 18.5%
-2	Light sedation	1, 3.7%

curium 0.2 mg kg^{-1} or rocuronium 0.6–1.0 mg kg^{-1} depending on the expected time of the procedure. The anaesthesia was maintained with sevoflurane (minimum alveolar concentration (MAC) of 1.0–1.4) and nitrous oxide 50% in oxygen using mechanical ventilation. During the maintenance of anaesthesia, the additional doses of 0.5–1 mcg kg^{-1} of fentanyl were given. Throughout the procedure patients were monitored according to standard procedures. To protect patients from hypothermia warming blankets were used. In all children emerging from anassthesia signs of delirium were not present.

After induction, 2.5 ml of peripheral blood was collected at certain points in time: 5 min (T_0), 10 min (T_1), 15 min (T_2), 30 min (T_3), 45 min (T_4), 60 min (T_5), as well as after 90 min (T_6) and 120 min (T_7), if time of procedure exceed 60 min. Plasma was obtained by blood centrifugation (4°C, 3.000 rpm, 10 min) and then stored at -80°C until use.

Drug and metabolite assay

Concentration of MDZ and its metabolite in plasma samples was assessed using validated high-performance liquid chromatography (HPLC, Agilent 1200 series, Waldbronn, Germany) coupled with a triple quadrupole mass spectrometer, equipped with an electrospray ionization source (Agilent 6410B, Wilmington, Delaware, USA), details were described previously [10]. Briefly, three reactions for each compound were recorded. Abselut Nexus (Agilent, USA) solid phase extraction columns (60 mg/ 3 ml) were used for MDZ and metabolite extraction, according to the manufacturer's procedure. Extraction recovery (% + SD) was 91.1 ± 3.5 and 86.8 ± 2.8 for MDZ and 1-OH-MDZ, respectively. Intraday precision (RSD, %) at 20 ng ml^{-1} standard was 5.3 and 7.2 for MDZ and its metabolite. Interday precision was 9.1 and 10.4 for MDZ and 1-OH-MDZ, respectively. The limit of quantification was 10 ng ml^{-1} for both analytes using 0.2 ml sample volume. The method was linear from 10 to 4000 ng ml^{-1}.

Population Pharmacokinetic Analysis

Population nonlinear mixed-effect modeling was done using NONMEM (Version 7.2.0, Icon Development Solutions, Ellicott City, MD, USA) and the gfortran compiler 9.0. NONMEM runs were executed using Wings for NONMEM (WFN720, http://wfn.sourceforge.net). The first-order conditional estimation with interaction (FOCEI) method was used. The self-written differential equations were solved using ADVAN6 PREDPP subroutines. The NONMEM data processing and plots were done in Matlab® Software version 7.0 (The MathWorks, Inc., Natick, MA, USA).

The minimum value of the NONMEM objective function (OFV), typical goodness-of-fit diagnostic plots, and the evaluation of the precision of pharmacokinetic parameter and variability estimates were used to discriminate between various models during the model-building process.

Pharmacokinetic Model

A standard two-compartment model with first order absorption was used to describe plasma MDZ concentrations:

$$\frac{dA}{dt} = -k_a A \; A(0) = D$$
$$\frac{dA_{MDZ,P}}{dt} = k_a A - \frac{CL/F}{V_P/F} A_{MDZ,P} - \frac{Q/F}{V_P/F} A_{MDZ,P} + \frac{Q/F}{V_T/F} A_{MDZ,P} \; A_{MDZ,P}(0) = 0$$
$$\frac{dA_{MDZ,T}}{dt} = \frac{Q/F}{V_P/F} A_{MDZ,T} - \frac{Q/F}{V_T/F} A_{MDZ,T} \; A_{MDZ,T}(0) = 0 \quad (1)$$

where t denotes time, A, $A_{MDZ,P}$ *and* $A_{MDZ,T}$ denotes MDZ mass in absorption, plasma and peripheral compartment; k_a denotes absorption rate constant; CL and Q denotes the metabolic and inter-compartmental clearance; F denotes bioavailability; and V_P and V_T denotes the volume of distribution of central and peripheral compartment, respectively. MDZ concentration equaled:

$$C_{MDZ,P} = \frac{A_{MDZ,P}}{V_P/F} \quad (2)$$

The MDZ metabolite, 1-OH-MDZ, concentration ($C_{1\text{-}OH\text{-}MDZ}$) was assumed proportional to MDZ concentration according to:

$$C_{1-OH-MDZ} = C_{MDZ,P}\frac{CL/F}{CL_{1-OH-MDZ}/f} \quad (3)$$

where $CL_{1\text{-}OH\text{-}MDZ}$ denotes 1-OH-MDZ clearance and f product of bioavailability and fraction of MDZ to 1-OH-MDZ metabolism. This equation was obtained assuming that elimination rate constant of 1-OH-MDZ is much higher than that of MDZ and assuming a very high absorption rate constant ($k_a >> k$) [10]. All the concentrations were in molar units.

Inter-individual variability (IIV) for the pharmacokinetic parameters was modeled assuming log-normal distribution:

$$P_i = \theta_P \exp(\eta_{P,i}) \quad (4)$$

where P_i is the individual parameter, θ_P is the typical value of this parameter in the population, and η_P is a random effect for that parameter with the mean 0 and variance ω_P^2.

Any j^{th} observations for i^{th} individual of MDZ ($C_{MDZ,P,ij}$) and 1-OH-MDZ ($C_{1\text{-}OH\text{-}MDZ,\,ij}$) were defined by:

$$C_{MDZ,P,ij} = C_{MDZ}(P_i, t_j)\cdot(1+\varepsilon_{prop,ij,MDZ}) \quad (5)$$

$$C_{1-OH-MDZ,ij} = C_{1-OH-MDZ}(P_i, t_j)\cdot \cdot(1+\varepsilon_{prop,ij,1-OH-MDZ}) \quad (6)$$

where $C_{MDZ,P}$ and $C_{1\text{-}OH\text{-}MDZ}$ are defined by basic structural model (Eq. 2 and 3) and $\varepsilon_{prop,ij,MDZ}$ and $\varepsilon_{prop,ij,1\text{-}OH\text{-}MDZ}$, represent the proportional residual random errors of MDZ and 1-OH-MDZ concentrations. It was assumed that ε is normally distributed with the mean of 0 and variances denoted by σ^2.

Covariance Analysis

The covariate search was performed by plotting individual estimates of the pharmacokinetics parameters against time-independent covariates (weight, age) to identify their potential effects. If a relationship was found, it was described by means of linear regression or power model (allometric relationship). The categorical covariate (gender) was included into the model based on indicator variables.

Specifically, the effect of body size on all the volume (V_C, V_T) and clearance (CL, Q) parameters was included a priori based on allometric scaling as follows:

$$P_i = \theta_P\left(\frac{BW_i}{70}\right)^K \exp(\eta_{P,i}) \quad (7)$$

where P_i denotes the individual value of volume and clearance term,, BW_i the individual body weight, 70 is a typical body weight of adult patients, and K is the exponent equal to 0.75 for clearance and 1 for volume of distribution [11].

The difference in the minimum of the NONMEM OFV obtained for two hierarchical models (likelihood ratio) is approximately $\chi 2$ distributed. During the covariate search the effect of each covariate was examined by adding an appropriate equation to the base model. The difference in OFV between models of 3.84 for one degree of freedom was considered to be statistically significant at $p < 0.05$ for the covariate to be included into the base model. This process was repeated until all significant covariates were added. Then, removing one covariate at a time performed backward elimination. The least important covariate was dropped from the model according to the OFV unless that difference in OFV was larger than 6.63 (corresponding to $p < 0.01$). The final model was established when no more covariates could be excluded from the model.

Model Evaluation

The model performance was assessed by means of predicted corrected Visual Predictive Check (pcVPC). The pcVPC was calculated based on 1000 datasets simulated with the final parameter estimates. The pcVPC plots were created by correcting the observed and simulated values for the average population prediction in the time-bin divided by population predictions for each observed and simulated value [12].

In this work the 10^{th}, 50^{th} and 90^{th} percentile were used to summarize the data and for VPC prediction. The pcVPC allows to compare the confidence inter-

vals obtained from prediction with the observed data over time. When the corresponding percentile from the observed data falls outside the 95% confidence interval derived from predictions this is an indication of a model misspecification. Since pharmacokinetics data deviated to some extent from nominal times, binning across time was done.

Bootstrap

Evaluation of model robustness was based on the non-parametric bootstrapping with 1000 replicates. From the bootstrap empirical posterior distribution, 90% confidence intervals (5^{th}–95^{th} percentile) were obtained for the parameters, as described previously [13].

Results

The analyzed data (n = 27) contained 344 MDZ and 1-OH-MDZ concentrations. The raw data are represented in **Figure 1**. Two IDs differed considerably from the other profiles and were excluded from the pharmacokinetic analysis. The brief summary of the analyzed patients is given in **Table 1**. Level of sedation by RASS, was assessed as satisfactory in the majority (55.6%) of patients (**Table 2**).

A two-compartment disposition model with first-order absorption was used to describe the available data. The 1-OH-MDZ concentrations were proportional to MDZ and a simplified direct relationship was used to describe the data, as described earlier [10].

Typical goodness-of-fit plots of the final model are presented in **Figure 2**. The individual and population prediction versus observed concentrations are relatively symmetrically distributed around the line of identity. The conditional weighted residuals versus time and versus individual predicted concentrations do not show any trend and are relatively uniformly distributed around the zero. The VPC plots stratified with respect to the type of measurements and moment during the

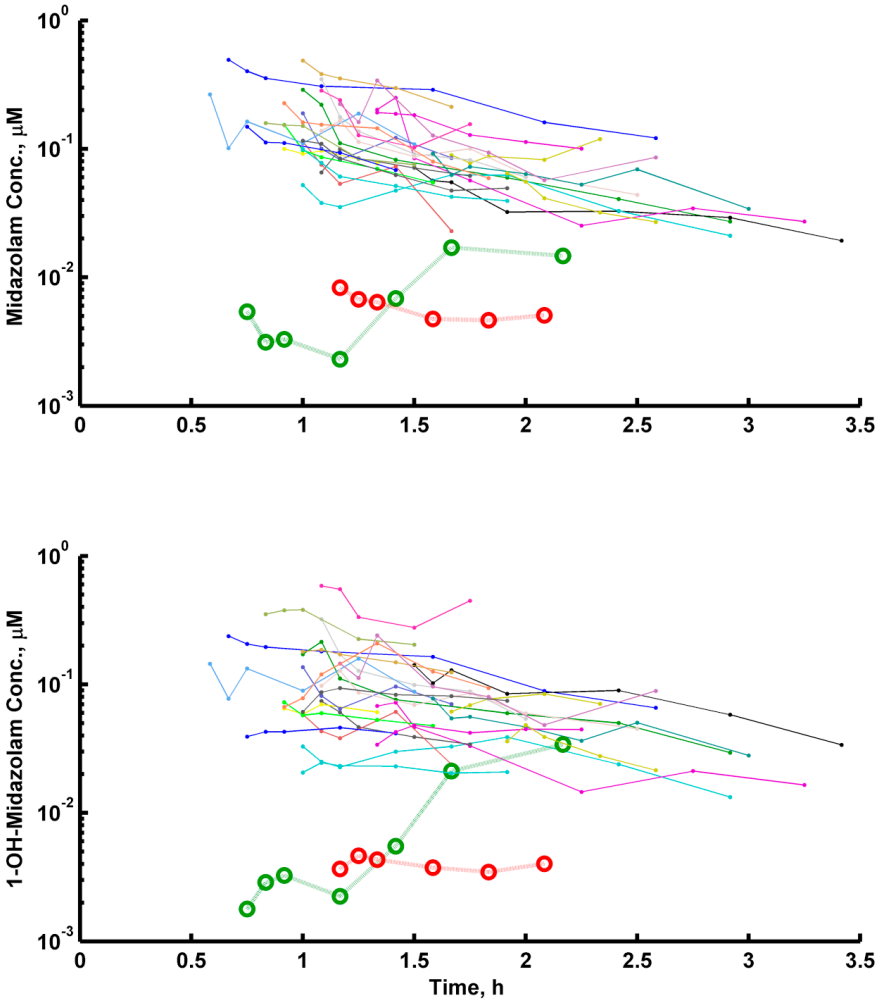

Figure 1. Individual (lines) MDZ and 1-OH-MDZ concentration time profiles. The 2 profiles (dotted line, open symbols) were not included in the analysis

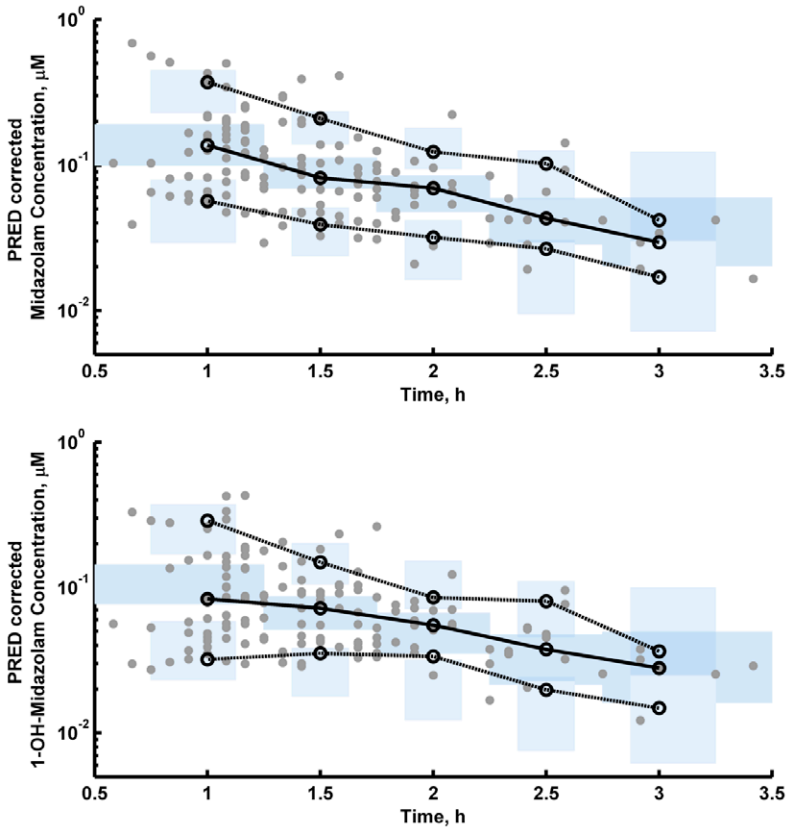

Figure 3. The prediction corrected Visual Predictive Checks (pcVPC). pcVPC plots show the simulation-based 95% confidence intervals around the 10th, 50th, and 90th percentiles of the pharmacokinetics data in the form of blue (50th) and gray (10th and 90th) areas. The corresponding percentiles from the prediction corrected observed data are plotted in black color. The prediction corrected raw data is presented as gray closed symbols

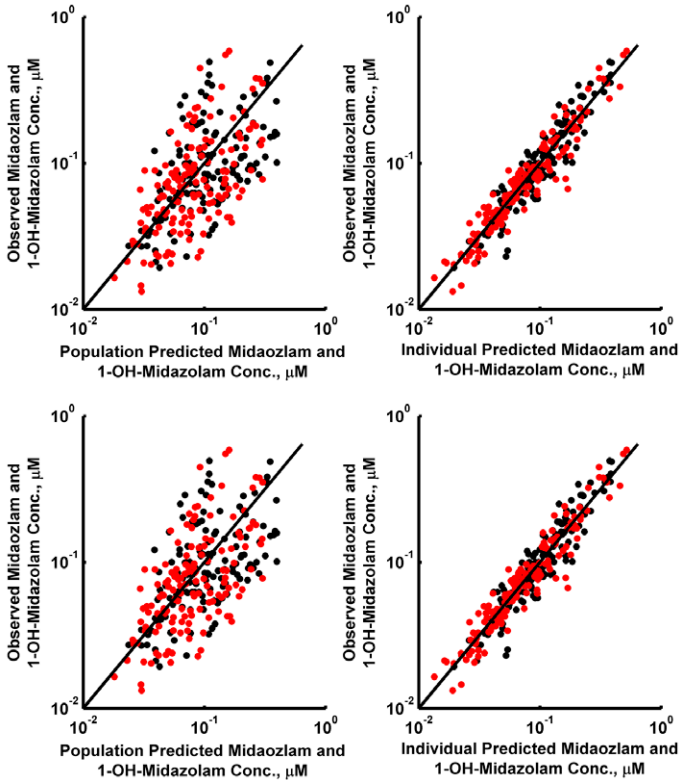

Figure 2. Goodness of fit plots: the observed versus the population predicted concentrations; the observed versus the individual population predicted concentrations; and conditional weighted residuals (CWRES) versus individual predicted concentrations and time. The black symbols denoted MDZ and red 1-OH-MDZ, respectively

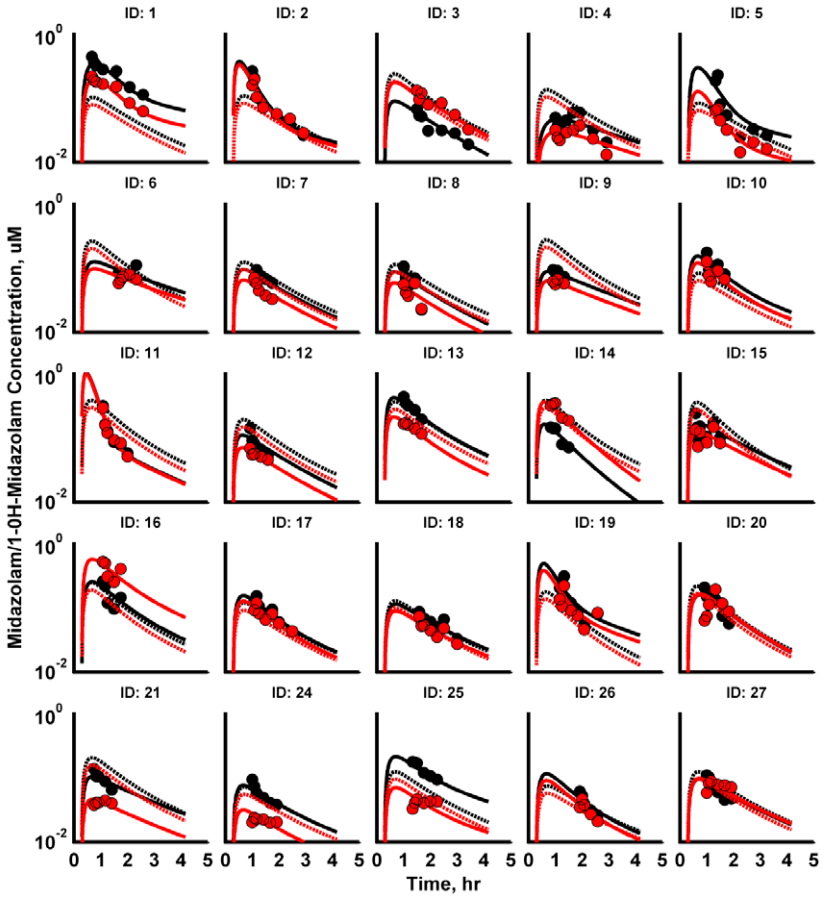

Figure 4. Experimental (red symbols), individual (black dotted) and population model predictions (red dotted) of MDZ (black) and 1-OH-midaozlam (red) concentrations

Table 3. Final model parameter estimates. 90% confidence interval (CI) of the parameter estimate derived from a nonparametric bootstrap analysis (n = 1000, unsuccessful = 79)

Parameter	Estimate	%RSE	Shrinkage%	Bootstrap median	Bootstrap 90% CI	
					Lower	Upper
k_a [1/h]	6.11 FIXED[a]	–	–	–	–	–
t_{lag} [h]	0.29 FIXED[a]	–	–	–	–	–
V_P/F [L]	176	18	–	191	122	280
Cl/F [L/h]	93.6	16	–	88.7	53.8	127
V_T/F [L]	67.8	64	–	90.7	23.5	493
Q/F [L/h]	27.0	35	–	30.7	12.3	71.6
$Cl_{1\text{-}OH\text{-}MDZ}/f$ [L/h]	123	15	–	114	67.7	157
$\omega^2_{VP/F,\ \%}$	93.1	34	1.0	116	80.8	165
$\omega^2_{CL/F,\ \%}$	59.4	26	3.6	68.8	43.6	119
$\omega^2_{CL\text{-}OH\text{-}MDZ,\ \%}$	58.9	54	3.9	63.5	40.2	104
$\omega^2_{Ka,\ \%}$	65 FIXED*	–		–	–	–
$\omega^2_{tlag,\ \%}$	15 FIXED*	–		–	–	–
$cor_{VP/F\text{-}\ CL1\text{-}OH\text{-}MDZ/f}$	0.82	38		0.88	0.66	0.99
$cor_{VT/F\text{-}\ CL1\text{-}OH\text{-}MDZ/f}$	0.59	45		0.75	0.46	0.97
$cor_{CL/F\text{-}CL1\text{-}OH\text{-}MDZ/f}$	0.63	28		0.75	0.48	0.92
$\sigma^2_{Prop,MDZ}$	0.24	10		0.238	0.192	0.277
$\sigma^2_{Prop,1\text{-}OH\text{-}MDZ}$	0.23	15		0.218	0.161	0.27

[a] Fixed based on work [21] Abbreviations: k_a – absorption rate constant; t_{lag} – lag-time; V_P/F – volume of central compartment of midazolam; Cl/F – oral clearance of midazolam; V_T/F – volume of peripheral compartment of midazolam; Q/F – intercompartmental clearance of midazolam; $Cl_{1\text{-}OH\text{-}Mid/f}$ – clearance of 1-OH-midazolam; $\omega^2_{VP/F}$ – inter-individual variance of VP/F; $\omega^2_{CL/F}$ – inter-individual variance of Cl/F; $\omega^2_{CL\text{-}1\text{-}OH\text{-}MDZ}$ – inter-individual variance of 1-OH-midazolam clearance; ω^2_{Ka} – inter-individual variance of ka; ω^2_{tlag} – inter-individual variance of Tlag; $cor_{VP/F}$ – CL-1-OH-MDZ/f – correlation between volume of central compartment of midazolam and clearance of 1-OH-midazolam; $cor_{VT/F}$- **CL-1-OH-MDZ/f** – correlation between volume of peripheral compartment of midazolam and clearance of 1-OH-midazolam; $cor_{CL/F}$- **CL-1-OH-MDZ/f** correlation between clearance of midazolam and clearance of 1-OH-midazolam; $\sigma^2_{Prop,MDZ}$ – residual variance for midazolam; $\sigma^2_{Prop,1\text{-}OH\text{-}MDZ}$ – residual variance for 1-OH-midazolam

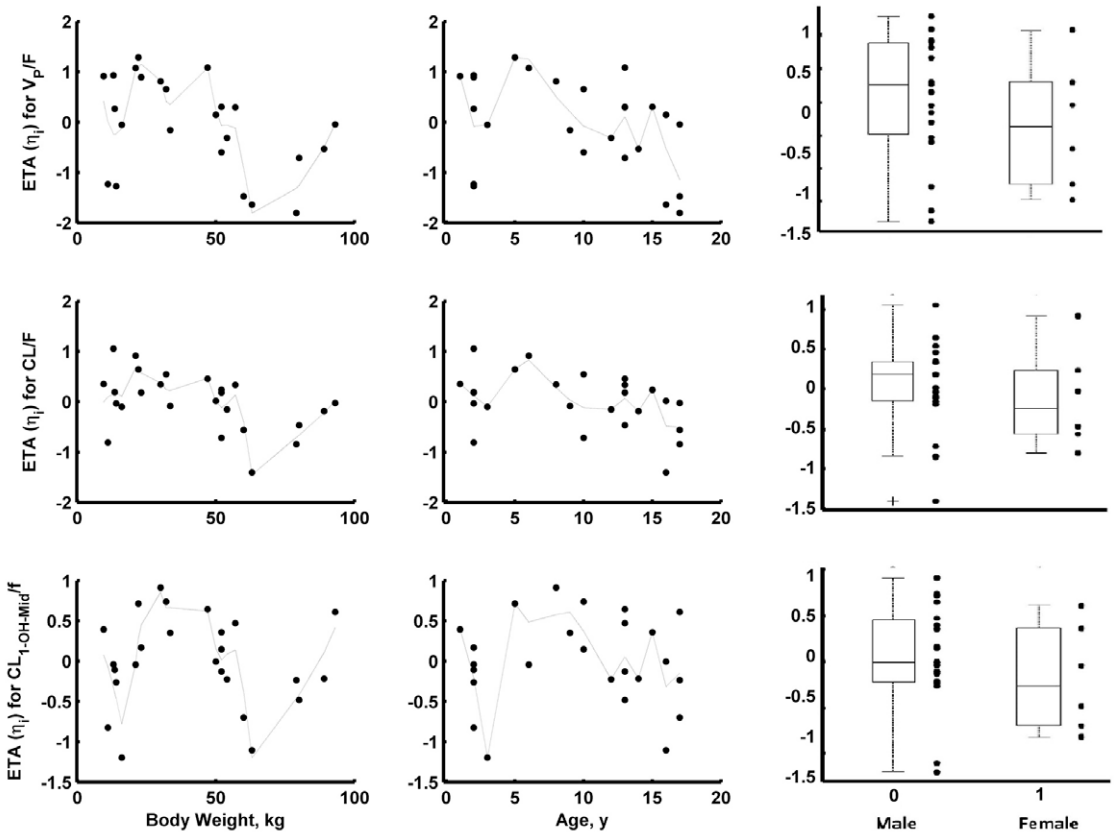

Figure 5. The individual estimates for eta (deviation of the individual estimate from the population mean) of the final pharmacokinetics/ parameters in relation to the patients' body weight, age and gender. The dotted line indicates the trend in the data (loess smooth)

infusion are presented in **Figure 3**. They all indicate that both the central tendency of the data and the variability at a particular sampling time were recaptured very well. Similarly, most of the individual predicted concentrations versus time profiles were very close to the experimental data as presented in **Figure 4**. **Table 3** shows parameter estimates of the final population pharmacokinetic model of MDZ along with their bootstrap estimates. All pharmacokinetics parameters, inter-subject, and residual error variances were estimated well with CVs lower than 64%.

The typical values of apparent (due to unknown bioavailability) volume of central and peripheral compartment were, respectively 176 and 67.8 L for MDZ. The apparent elimination and inter-compartmental clearance equaled 93.6 L h^{-1} and 27 L h^{-1}. For 1-OH-MDZ the apparent clearance equaled 123 L h^{-1}. The inter-individual variability was high and equaled about 93% for volume of central compartment and 60% for clearance, respectively.

The effect of body weight on MDZ and 1-OH-MDZ pharmacokinetics was well explained by an allometric relationship with theoretical exponents. Age and gender were not found to be independently significant covariates in this study. The relationship between the individual estimates for eta (deviation of the individual estimate from the population mean) of the *CL/F, VP/F*, and $CL_{1\text{-}OH\text{-}MDZ}$ and the individual values of the covariate (eta-plots) are presented in **Figure 5**. The lack of any trend in the data indicates that the above mentioned covariates do not explain the remaining unexplained ones between patients variability for *CL/F*, V_p/F, and $CL_{1\text{-}OH\text{-}MDZ}$.

Discussion

Development of MDZ pharmacokinetic model is pivotal for predicting drug response and determining appropriate dosing as a premedication in patients who undergo surgical procedures. It is also important to establish which factors are responsible for inter-individual variations in MDZ clearance. In this study the population pharmacokinetic model was successfully developed to describe the time course of MDZ and 1-OH-MDZ concentrations in paediatric patients. The influence of age, gender and weight on MDZ and 1-OH-MDZ clearance was also investigated. We were unable to show any statistically significant

differences in the studied population due to the gender. No maturation could be identified, that for this weight was included as covariate according to allometric scaling principles. The allometric principle well accounted for the body weight effects on MDZ and 1-OH-MDZ pharmacokinetics parameters.

Based on the literature data, inter-individual variation in the pharmacokinetics of MDZ, especially in its clearance, may result from differences in many factors such as age [5–7, 14], weight [15, 16], disease occurrence [17] as well as ethnicity/genotype [16, 18]. Some of studies on age-related changes in pharmacokinetics of MDZ administrated intravenously have demonstrated altered pharmacokinetics parameters depending on the patient's age. Prolonged $t_{1/2}$ and a decreased weight-corrected clearance of MDZ were observed in neonates [5, 6] as well as MDZ clearance was higher in children aged 3 years and older, than in infants and children from 1 to 2 years [7]. On the other hand, some studies on weight-corrected oral MDZ clearance have not revealed age-related changes [19, 20]. Moreover, it was shown that weight-adjusted MDZ clearance decreases according to the power-low relationship with body weight [21]. Increased weight-normalized MDZ clearance in children of lower weight is sometimes explained by their greater liver volume relative to total body weight (4% of the body in 1-year-old children compared to 2.5% in adults) [22, 23] or by higher concentration of catalytically active cytochrome P450 3A4 (CYP3A4) per gram liver weight in children. Despite the underlying mechanism all the literature reported findings consistently suggest that age affects MDZ clearance up to second year of life, and later changes in pharmacokinetics of MDZ can be well explained by body weight differences [4].

In a systematic review, in which the extent of inter-individual variation in MDZ clearance in children and factors responsible for this variation were determined, it was shown that variation in MDZ clearance is greatest in critically ill children and neonates [24, 25]. There are several factors that may affect the pharmacokinetics of drugs in critically ill patients including: hypoxia, shock, systemic inflammatory responses, stress, changes in diet, endocrine changes and other drugs [25–28]. Moreover, it was determined that the degree of inter-individual variation in these patients is far greater than the variation in administered doses of MDZ. As a result, it is likely that some of these paediatric patients may receive inadequate dose of MDZ and as a consequence be underdosed or overdosed with this sedative [24]. Two patients were removed from the analysis, as they had considerably different pharmacokinetics that could not be associated with available covariates. The presence of outlying concentration-time profiles confirms the existence of large inter-individual variability in MDZ metabolism. The variability may be determined by many factors, including genetic, environmental and demographic ones. Excluded patients received the same dose of MDZ (7.5 mg), were of the same age, one was a man and one was a woman weighing 69 kg and 56 kg, respectively. Level of sedation assessed according to the Richmond Agitation-Sedation Scale (RASS) had the value of 0 (described as alert and calm, patient spontaneously pays attention to caregiver) for both patients. It could indicate that the observed differences in pharmacokinetics-profiles are more likely caused by other mechanism, like patients noncompliance or delayed gastric emptying.

Moreover, in this age group of patients (1–17 years) effect of age and gender is minimal and if it would be visible, it can be well explained by differences in body weight. There may be a number of factors contributing to the variation in MDZ pharmacokinetics, thus further studies are necessary to determine the influence of particular covariates (demographic, genetic, environmental, diseases occurrence) on pharmacokinetics of MDZ.

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Correspondence address:
Alicja Bartkowska-Śniatkowska
Department of Paediatric Anaesthesiology and Intensive Therapy
Poznan University of Medical Sciences
email: asniatko@ump.edu.pl

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Depression in seniors vs. their nutritional status and nutritional knowledge

Rafał W. Wójciak[1], Ewa Mojs[1], Halina Staniek[2], Katarzyna Marcinek[2], Ewelina Król[2], Joanna Suliburska[2], Zbigniew Krejpcio[2]

[1] Department of Clinical Psychology, Poznan University of Medical Sciences, Poland
[2] Food Hygiene and Toxicology Chair, Department of Human Nutrition and Hygiene, Poznan University of Life Sciences

ABSTRACT

Aim. There is a limited amount of data on depression in elderly people and even less is known about the relationship between nutritional status and nutritional habits, and mood disorders in this group. The foregoing paper investigates this relationship and evaluates the family status of seniors with depression.
Material and methods. The research was conducted on a group of 85 seniors in the ages of 60–91 years. The depression symptoms, nutritional knowledge and nutritional status were investigated and their relationship in elderly women.
Results. Depression was present in 27% of women. Seniors with depression had a significantly lower BMI and a larger number of them had better nutritional knowledge than the rest of women. Women with the lowest BMI were statistically younger, had better nutritional knowledge and higher level of depression than women with normal BMI. There is a relationship between depression and family status of seniors. A significantly larger number of single women suffered from depression (50%) in comparison with women in relationships (25%) and single widows (23%).
Conclusions. Research showed that there is a relationship between symptoms of depression in elderly women with nutrition and family status. Extending research in order to evaluate nutrition disorders in seniors, which influence their mood disorders seems justified.

Key words: women, seniors, depression, nourishment.

Introduction

Civilization progress, particularly in the field of medicine, treatment and illness prevention, led to the increase of life expectancy [1, 2]. At the same time due to the recent decrease in the population growth in well-developed countries the number of elderly people in the general population increases [1, 2].

Demographic data indicate that 25% of people worldwide and 50% of people in Europe will reach the age of 65 in the next 50 years [2–4]. 14% of people living in Poland in 2013 have reached 65 [2] and it is estimated that this number will reach 21% within the next 10 years [4, 5].

With age the human state of health slowly deteriorates, which means that old age is very often characterized by somatic multisymptom illnesses brought about both by pathological symptoms or resulting from the physiological process of aging [6–8]. Among the main causes of disorders in the elderly age there are problems with nutrition, caused by changes in the metabolism and problems with chewing and digesting the food [8, 9]. One of the most difficult challenges for the elderly people is changing their eating habits, which is necessary if they want to stay healthy [8, 10, 11]. A different structure of food, different methods of preparing food, regular meals and their taste are

all elements that should be considered in their everyday diet and which require proper knowledge about healthy nutrition. There is little information about the seniors' level of knowledge about proper nutrition. However it should be mentioned that nutritional preferences of the elderly are often the wrong ones. They may lead to health disorders, such as overweight and obesity, high blood pressure, heart diseases, hypercholesterolemia, etc. [10, 12]. On the other hand scientific research fails to mention that more and more elderly people are either over- or underweight. This may be caused by disorders in digesting and absorbing food, as well as by problems with chewing, lack of taste, new cooking methods, which the elderly find unacceptable. Psychological factors should also be considered. Peoples' attitude towards old age, loneliness or the need to face their disabilities are the source of fear, paranoid disorders, dementia or depression [1, 3, 5, 13, 14].

Depression is a group of emotional disorders that may intensify when accompanied by somatic disorders. Depression is characterized by the strong feeling of fear, lowering the mood and physical activity. It is estimated that depression, next to dementia, is the most commonly diagnosed mental disorder in the elderly. It is diagnosed in about 15% of people over 65 [2, 7]. Depression in the elderly people often leads to disability, impairing their quality of life and causing premature death, as well as suicides [1, 2, 15]. It is mainly caused by loneliness after losing the life partner, an empty nest syndrome, the feeling of uselessness or suffering caused by disability [1, 2, 15, 16]. In the last few years it has also been found that depression may be related to quantitive and qualitative malnutrition [17–20]. Anaemia caused by iron deficiency may particularly influence certain mood disorders [6, 17, 19]. Even 65% of elderly people may have low iron levels, thus suffering from anaemia [6]. So far scientists have not been particularly interested in investigating the relationship between depression in an elderly age with proper nourishment and the level of knowledge about nourishment. It seems that it may have an influence on initiating the foregoing disease and on its prevention.

Aim

The aim of this preliminary research was an attempt to evaluate the occurrence of depression in elderly women and its relations to their nutritional status and nutritional knowledge.

Materials and methods

Research was conducted on a group of 85 seniors in the ages from 60 to 91 (average 73.3 ± 9.0 years) residing in big cities (over 50 thousand inhabitants) in the Wielkopolska area. People participating in the study were divided into three groups based on their age (60–70 years – 37 women, 70–80 years – 25 women and above 80 years – 2 women). After explaining the purpose of research and obtaining the consent of the participants, they were first weighed and measured (medical scales with accuracy to 1 cm and 1 g respectively). Anthropometric measurements were used to calculate the Quetelet's index – mass to height (Body Mass Index; BMI), based on which the nourishment condition of the participants was evaluated. The proper value was between 18.5 and 24.9 kg/m^2 [17]. Next, seniors were asked to fill out a questionnaire which contained the GAROTA nutritional knowledge test [21]. 42 questions with three levels of difficulty, which represented all sections were randomly selected from all the questions in the survey. Taking into consideration the age of the applicants, only multiple choice questions were selected and evaluated in the scale from 0 to 1. The average level of knowledge was determined as 50–70% of proper answers (22–30 points). Depression was also evaluated based on the Geriatric Depression Scale (GDS). People with the score above 10 points in the GDS test, which indicated light depression and above 20 points – indicating deep depression, underwent an individual psychological diagnosis based on the ICD-10 depression criteria, which helps to determine the occurrence and level of depression [15, 16]. To evaluate the family status women were divided into those living alone (including single women that were never in a relationship and widows living alone), and those living with partners.

To evaluate the significance of differences between the average values a t-Student test was used, while distribution was evaluated with the $\chi 2$ test.

Results

Table 1 presents average results of the evaluated parameters. The average BMI index in seniors was within normal range (24.1 ± 4.5 kg/m^2; 16.5 – 36.8 kg/m^2) and was similar in particular age groups. The average result of the GDS test showed no signs of depression in the examined groups (8.2 ± 6.2; 0–24), regardless of age. The seniors' level of nutritional knowledge was average (GAROTA test – 24.2 ± 3.6; 17–33). Nutritional knowl-

Table 1. Average results of analysed parameters in seniors

		Age (years old)	BMI (kg/m^2)	Garota test results	GDS
Total n = 85		73.3 ± 9.0 (60.0–91.0)	24.1 ± 4.5 (16.5–36.8)	24.2 ± 3.6 (17.0–33.0)	8.2 ± 6.2 (0.0–24.0)
Group < 70 y.o. n = 37 (44%)		64.9 ± 2.7 (60.0–69.0)	24.7 ± 4.5 (16.5–36.8)	24.9 ± 4.3^b (18.0–33.0)	9.0 ± 5.4 (2.0–23.0)
Group 70–80 y.o. n = 25 (29%)		74.5 ± 2.7 (70.0–79.0)	24.0 ± 3.5 (16.5–29.4)	24.6 ± 2.6^b (18.0–30.0)	7.6 ± 6.9 (0.0–24.0)
Group > 80 y.o. n = 23 (27%)		85.5 ± 3.3 (80.0–91.0)	23.4 ± 5.3 (17.1–35.0)	22.9 ± 2.9^a (17.0–30.0)	7.7 ± 6.9 (0.0–24.0)
Without depression n = 62 (73%)		73.5 ± 8.9 (60.0–91.0)	25.0 ± 4.2^b (17.6–36.8)	24.0 ± 3.7 (17.0–33.0)	5.0 ± 2.8 (0.0–10.0)
With depression n = 23 (27%)		72.8 ± 9.3 (61.0–91.0)	21.8 ± 4.3^a (16.5–29.3)	25.0 ± 3.2 (20.0–32.0)	16.9 ± 4.4 (12.0–24.0)
BMI < 18,5 n = 12 (14%)		71.3 ± 8.8^a (61.0–90.0)	17.7 ± 1.0 (16.5–18.5)	25.5 ± 3.1^b (21.0–32.0)	13.2 ± 6.8^c (4.0–24.0)
BMI 18,6–24,9 n = 36 (42%)		76.0 ± 9.5^b (60.0–91.0)	22.0 ± 1.9 (19.1–24.9)	23.7 ± 2.6^a (17.0–28.0)	8.8 ± 6.5^b (2.0–24.0)
BMI > 25,0 n = 37 (43%)		71.3 ± 8.0^a (61.0–85.0)	28.3 ± 2.6 (25.5–36.8)	24.3 ± 4.4^{a,b} (18.0–33.0)	6.0 ± 4.7^a (0.0–16.0)
Nutritional knowledge	Low n = 18 (20%)	74.7 ± 10.0^b (63.0–90.0)	25.4 ± 4.0^b (17.1–31.2)	19.4 ± 1.4 (17.0–21.0)	6.2 ± 5.1 (0.0–16.0)
	Average n = 59 (69%)	73.7 ± 8.5^b (60.0–91.0)	23.4 ± 4.1^a (16.5–33.6)	24.7 ± 1.9 (22.0–28.0)	8.7 ± 6.4 (0.0–24.0)
	High n = 8 (9%)	67.2 ± 8.4^a (61.0–85.0)	26.3 ± 6.9^b (16.7–36.8)	31.2 ± 1.4 (30.0–33.0)	9.6 ± 7.2 (2.0–24.0)
Family status	Singles n = 20 (23%)	69.8 ± 4.9^a (64.0–84.0)	23.3 ± 4.4 (16.5–29.1)	23.3 ± 3.7^a (18.0–30.0)	12.1 ± 7.7^b (0.0–24.0)
	With partners n = 26 (31%)	67.5 ± 6.1^a (60.0–79.0)	24.1 ± 4.8 (16.7–36.8)	26.0 ± 3.9^b (19.0–33.0)	6.8 ± 4.0^a (0.0–15.0)
	Widows n = 39 (46%)	79.0 ± 8.8^b (62.0–91.0)	24.5 ± 4.3 (17.1–35.0)	23.5 ± 2.9^a (17.0–30.0)	7.2 ± 6.0^a (0.0–24.0)

a, b, - the significant differences between means

edge of the oldest seniors with their result in the GAROTA test ($p < 0.05$) on the level of 22.9 ± 2.9 (17–30) was slightly smaller as compared to seniors from other age groups (average of approx. 24.7 ± 3.0; 18–33).

Depression was diagnosed in 27% of the participants, while most seniors complained of some depression symptoms (**Tabela 1**). 6 women were diagnosed with moderate depression. There were no statistical differences between women with diagnosed depression and the rest of seniors in terms of their age and level of nutritional knowledge. Statistically lower ($p < 0.01$) average BMI (21.8 ± 4.3 kg/m^2) was observed in women with depression than in the rest of women (25.0 ± 4.2 kg/m^2).

A similar percentage of seniors examined (approx. 40%) was overweight or had a proper BMI. A lower BMI was observed in 14% of women, who had greater nutritional knowledge (mean 25.5) and a GDS result (mean 13.2) that would indicate mild depression as compared with women with proper BMI and overweight (women who attained 23.7 and 24.3 points in the GAROTA test respectively, and 8.8 and 6.0 in the GDS). Overweight women showed smaller symptoms of depression than women with proper BMI and underweight ($p < 0.05$ and $p < 0.01$ respectively).

Analysis of the nutritional knowledge showed that about 70% of respondents had a medium level of such knowledge and in only 9% of them this level of knowledge was high. People with high results in the GAROTA test were significantly younger that those with average and low results ($p < 0.05$) and had a bigger BMI ($p < 0.05$) as compared to people with average nutri-

Table 2. Percentage distribution of the examined population with or without depression in relation to the nutritional status, nutritional knowledge and family status

		With depression	Without depression
Nutritional status as a BMI	Below normal	22	8
	Normal	52	39
	Overweight	26	53
Statistic		$\chi^2 = 17.62$ $p < 0.001$	
Nutritional knowledge	Low	44	55
	Average	56	42
	High	0	3
Statistic		$\chi^2 = 6.22$ $p < 0.05$	
Family status	Singles	44 (50*)	16 (50*)
	Women with partners	17 (25*)	36 (75*)
	Singles widows	39 (23*)	48 (77*)
Statistic		$\chi^2 = 20.81$ $p < 0.001$	

(*) the frequency occurrence of depression in singles, singles widows and women living with their partners ($\chi 2 = 20.58$; $p < 0.001$)

tional knowledge and a BMI similar to people with low test results. There were no differences between the groups with different levels of nutritional knowledge to the extent of moderate depression symptoms.

Table 2 presents a percentage distribution of seniors with and without diagnosed depression in relation to the BMI and nutritional knowledge. There is a statistically significant difference between the groups with and without depression in both analyzed parameters ($p < 0.001$ and $p < 0.05$ respectively). A greater number of seniors with depression was underweight (22%) as compared to women without emotional disorders (8%). Most people not diagnosed with depression were overweight (53%), while only 26% of people with depression had a bigger BMI.

The majority of seniors with depression had an average (56%) and low (44%) level of nutritional knowledge, while most seniors without depression (55%) had a low and average level of knowledge (42%). People with high level of nutritional knowledge were found only in this group.

The foregoing paper also examines the influence of family status on the analysed parameters. 46% of seniors were widows living alone (**Table 1**), which had an average life expectancy (79.0 ± 8.8 years) as compared to single women (respectively: 23%; 69.8 ± 4.9 years) and women in relationships (31%; 67.5 ± 6.1 years, respectively). The analysis of nutritional knowledge showed that women living with partners have greater knowledge ($p < 0.01$) than single women or widows living on their own (average result of GAROTA test: 26.0 ± 3.9 vs. approx. 23.0 ± 3.5, respectively). The biggest statistical difference ($p < 0.001$) in expressing depression diagnosed with a GDS and confirmed with an individual diagnosis was found between single ladies and seniors living with their partners and widows (12.1 ± 7.7 vs. approx. 7.0 ± 5.0, respectively). This relationship was confirmed by the analysis of the percentage distribution of people with diagnosed depression in relation to their family status and people with various family status – in relation to diagnosed depression (**Table 2**). Among seniors with diagnosed depression 83% (44 vs. 39%, respectively) were women – single women and widows. Widows and seniors in relationships showed no signs of depression (48 vs. 36% respectively). Presented distribution differences were extremely significant ($p < 0.001$). Analysis of the occurrence of depression in relation to a person's family status ($p < 0.001$) confirmed that 50% of single seniors were diagnosed with depression, while in case of women in relationships and widows depression was diagnosed in only 25% of them.

Discussion

Examining depression in elderly people is extremely difficult due to the insufficient number of diagnostic criteria and multiple factors of the disease to be considered. The causes of depression in seniors are related to decreased physical fitness and to how seniors themselves describe the change in their quality of life. Some authors [1, 2, 5] declare that depression is the

most common mental disorder of the elderly. However evaluation of its frequency varies from 4 to 40%. For example, Babiaczyk et al. [5] analyzed a group of over 200 people over 65 and found that over 50% of the examined group showed signs of depression. They did not show statistical differences between light depression in women and men (approx. 40%), whereas a greater number of women (14%) suffered from deep depression as compared to men (approx. 2%). Different data can be found in the research of Pacjan et al. [2], which showed that 19% from among 70 examined people suffered from depression. Different data was obtained from the research of Walkiewicz et al. [6], where light depression was observed in 75% of examined women. However, the latter data were based only on examining 16 seniors.

In the foregoing paper depression was diagnosed in 23% of examined seniors. Intensified depression, based on the average number of points in the depression scale, did not depend on the age of the examined but significantly differed depending on the state of nutrition evaluated based on the BMI and on the method of nutrition, evaluated based on the level of nutritional knowledge. Intensified mood disorders in seniors with the lowest BMI were statistically more frequent than in women with proper BMI and twice as high as compared to women with excess weight. Significant intensification of depression was also observed in women with proper body weight rather than in overweight women. Analysis of the answers in the nutritional knowledge test showed that elderly people with the lowest body weight had the highest nutritional knowledge. No significant correlation between the points obtained in the test and intensity of depression was observed, whereas there was a significant, yet small ($p < 0.05$), negative correlation between women's BMI and the number of points in the depression scale.

Scientific papers often present depression as a disease that accompanies nutrition disorders, particularly anorexia [17, 19, 20, 22]. Literature presents no information about anorexia or other nutritional disorders in seniors. Researchers mostly concentrate of the co-existence of overweight and obesity in the elderly age as disorders resulting from the change of metabolism in seniors, as well as from the risk factors of many diseases diagnosed in the old age [8, 13]. Correlation between BMI, depression and nutritional knowledge presented in the foregoing paper proves that nutritional disorders in seniors should be further investigated. The work of Walkiewicz et al. [6] evaluated the co-existence of depression and anaemia caused by iron deficiency. Although the authors showed no statistical relation between the foregoing parameters, possibly due to a small number of examined seniors, they were able to diagnose anaemia in 67% of women. Many papers present a relation between anaemia and iron deficiency with not eating properly, which is characteristic for eating and mood disorders, including depression [17, 18, 19, 22].

Analysis of population distribution seems to confirm a strong relationship between the level of nourishment in the elderly people and depression. Seniors with such disorders are neither over- or underweight (approx. 20%), while 50% of women with no signs of depression were overweight and only 8% were underweight. The majority of seniors with depression also showed higher nutritional knowledge. Once again the broadly discussed issue that nutritional knowledge does not always mean good nutrition is confirmed [11, 21, 22]. This is particularly the case with people suffering from eating disorders, who often have proper knowledge, which is significantly higher than in the rest of society.

It is also worth mentioning that with elderly people eating disorders may not be similar to those diagnosed in young people. Our own clinical research shows that aversion to food, particularly in the elderly age, is characteristic for people who have problems with chewing and swallowing due to the dysfunction of taste buds, who suffer from gastrointestinal disorders and for some people with dementia. Koczorowska and Jundziłł-Bieniek [9] emphasized the relations between depression and the condition of teeth, indicating that seniors with depression find it hard to adapt to prosthetic restauration and how missing teeth influence the initiation of a disease.

The foregoing paper also investigated the influence of family status on mental disorders, as well as the difference in family status between seniors with and without depression. Pursuant to literature data [22, 23] most significant symptoms of depression were visible in lonely women as compared to seniors living with partners or widows. Even though among women with depression there is the same number of women who were never in a relationship and widows living alone, the number of singles with depression was significantly higher than with women from other subgroups (50% vs. 20%).

To sum up, the observed relationship between depression and low body weight, therefore low BMI and higher nutritional knowledge may suggest that seniors suffer from restrictive eating disorders that

may be observed in other age groups. Therefore, it seems that conducting research aimed at determining detailed causes of this issue and implementing proper preventive programs. Interdisciplinary cooperation of gerontologists, psychologists and dieticians is extremely important and may improve the quality of life of the elderly people.

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Correspondence address:
Rafał W. Wójciak
Department of Clinical Psychology
Poznan University of Medical Sciences
70 Bukowska Str, 60-812 Poznań, Poland
phone/fax: +48618547274
email: rafwoj@ump.edu.pl

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The Finnish Diabetes Risk Score (FINDRISC) and increased body weight

Joanna Pekar[1], Rafał Mazur[1], Małgorzata Kozilewicz[1], Aleksandra Jóźwiak[1], Anna Olszewska[2], Katarzyna Skórzyńska-Dziduszko[2]

[1] Students' Scientific Association at the Chair and Department of Human Physiology, Medical University of Lublin, Poland
[2] Chair and Department of Human Physiology, Medical University of Lublin, Poland

ABSTRACT

Introduction. The Finnish Diabetes Risk Score (FINDRISC) assesses the 10-year type 2 diabetes risk in adults by identifying individuals with overweight or obesity, inadequate physical activity, poor nutrition, or a family or personal history of hyperglycaemia.
Aim. The objective of the study was to analyse the effect of FINDRISC components, particularly overweight/obesity, on the total FINDRISC score of randomly selected individuals.
Material and methods. The study was conducted in 2015 on 91 individuals – 45 women and 46 men. We determined FINDRISC score and measured blood pressure twice. The results were analysed in STATISTICA 10 at $p < 0.05$.
Results. Thirty subjects (32.97%) were overweight (BMI 25–29.9 kg/m^2) and 12 (13.19%) were obese (BMI >= 30 kg/m^2); 25 (27.47%) had high waist circumference (M: 94–102 cm; F: 80–88 cm) and 24 (26.37%) abdominal obesity (M: > 102 cm; F: > 88 cm). Individuals with overweight/obesity, high waist circumference or abdominal obesity had significantly higher FINDRISC scores than those with normal body weight and waist circumference. Obese individuals showed a strong tendency ($p = 0.06$) towards higher FINDRISC scores than overweight individuals, but no similar difference was noted between high waist circumference and abdominal obesity. Overweight and obese subjects had significantly higher blood pressure, but with no difference between them. Individuals with abdominal obesity, but not those with high waist circumference, had significantly higher blood pressure.
Conclusions. Diabetes risk is increased by high waist circumference, but does not continue to increase with waist circumference, whereas in the case of BMI the risk gradually increases. BMI influences blood pressure more than waist circumference does.

Keywords: overweight, obesity, waist circumference, FINDRISC.

Introduction

Type 2 diabetes is a progressive condition in which the body becomes resistant to the normal effects of insulin and/or gradually loses the capacity to produce insulin.

The number of patients with type 2 diabetes is continuously rising. The International Diabetes Federation (IDF) publishes a yearly report on the incidence of diabetes worldwide and in individual countries. The most recent report indicates that 415 million people suffered from diabetes worldwide in 2015, including 6.2% of the population in Poland [1].

The risk factors for type 2 diabetes include overweight and obesity. The problem of obesity affects all segments of society. According to the Central Statistical Office of Poland, in 2009 about 36.4% of the

Polish population was overweight and 15.8% were obese. This problem is beginning to affect people at an increasingly early age, including children and teenagers [2].

The Finnish Diabetes Risk Score (FINDRISC) questionnaire is a screening tool used to estimate the 10-year risk of type 2 diabetes in adults.

The scale is used to identify individuals with the following risk factors of diabetes: age, overweight or obesity, a low level of physical activity, poor nutrition, a family or personal history of hyperglycaemia, or use of anti-hypertensive medication. FINDRISC consists of eight questions about these risk factors. Every question gives a score in relation to how much it predicts the risk of T2DM. The total score from the questionnaire predicts the future risk for T2DM within 10 years. The maximum score possible to get is 26. Identification by FINDRISC of high-risk individuals can be followed by educational intervention, which has been shown to reduce the incidence of diabetes and prevent the development of complications of this disease [3, 4].

Since the FINDRISC score includes both the body mass index and waist circumference to evaluate the type 2 diabetes risk, an interesting question arises as to how strongly these factors may affect FINDRISC score.

The objective of the study was to analyse the effect of FINDRISC components, particularly overweight/obesity, on the total FINDRISC score of randomly selected individuals.

Material and methods

The study was carried out in 2015 by medical students during the 12th Lublin Science Festival on a group of 91 individuals – 45 women (F) and 46 men (M). Participation in the study was voluntary and anonymous. The participants were selected randomly. Participants were divided according to gender, BMI and waist circumference. The FINDRISC score was determined for the subjects and their blood pressure (BP) was measured twice. A risk score from 0 to 7 indicates a low risk of type 2 diabetes (an estimated 1 in 100 individuals in this group will develop diabetes within 10 years); 7–11 indicates a slightly increased risk (about 1 in 25 will develop diabetes within 10 years); 12–14 a moderate risk (1 in 6 individuals), and 15–20 a high risk (1 in 3). When the score exceeds 20, the risk is considered to be very high (an estimated 1 of every 2 people in this group will develop type 2 diabetes within 10 years) [5]. The values for the parameters analysed were presented as arithmetic mean, standard deviation, minimum and maximum values, lower and upper quartiles, and median. The Shapiro-Wilk test was used to assess the normality of distribution of parameters, the chi-squared test to determine whether there was a significant difference between the expected frequencies and the observed frequencies in categorical data categories, and the Kruskal-Wallis H test to compare the type of distribution and variance homogeneity between more than two groups. A significant Kruskal-Wallis H test was followed by the Dunn-Bonferroni post hoc method, which was used to compare the difference in the sum of ranks between columns with the expected average difference. A p value of less than 0.05 was considered statistically significant. The data were analysed using STATISTICA 10.0 software (StatSoft, USA). All procedures involving participants were approved by the local Research Ethics Committee (KE-0254/71/2011).

Results

Thirty subjects (32.97%) were found to be overweight (BMI 25–29.9 kg/m^2) and 12 (13.19%) were obese (BMI >= 30 kg/m^2). High waist circumference (M: 94–102 cm; F: 80–88 cm) was noted in 25 (27.47%) subjects and abdominal obesity (M > 102 cm; F > 88 cm) in 24 (26.37%).

Individuals with abdominal obesity were statistically significantly older (the Kruskal-Wallis H test, $p < 0.05$). In the case of BMI no statistically significant relationship with age was observed, although BMI showed a tendency to increase with age (the Kruskal-Wallis H test, $p = 0.08$).

Obese individuals showed a tendency (the Kruskal-Wallis H test, $p = 0.06$) towards higher FINDRISC scores than overweight individuals (**Figure 1**).

In terms of the FINDRISC score no statistically significant difference was found between subjects with high waist circumference and those with abdominal obesity (**Figure 2**).

Among individuals with normal BMI there were four with abdominal obesity, whereas all obese subjects also had abdominal obesity.

Subjects with BMI > 30 kg/m^2 statistically significantly more often reported taking antihypertensive drugs than respondents with lower BMI (the chi-squared test, $p < 0.05$), but this association was not found in the case of individuals with high waist circumference. When the 29 individuals that declared taking

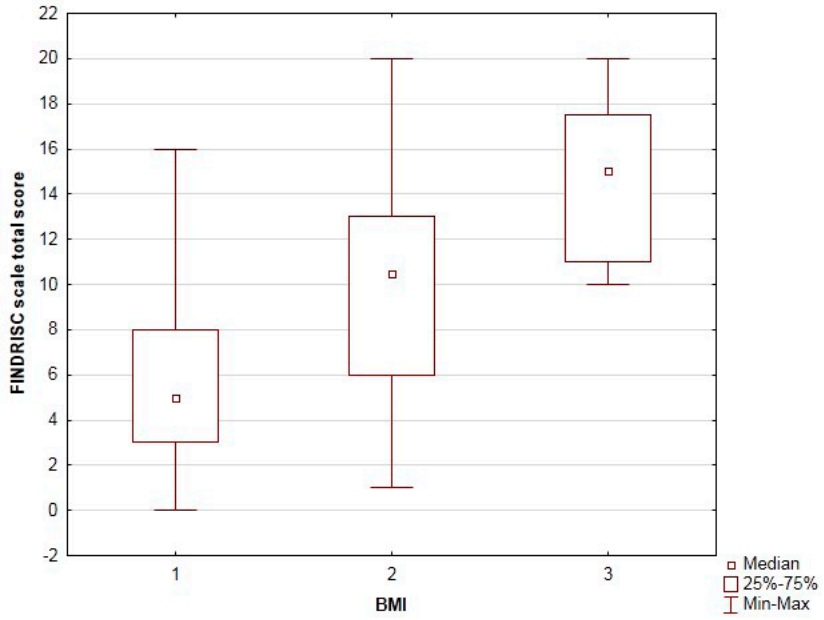

Figure 1. FINDRISC score in subjects with normal BMI < 25 kg/m² (1), overweight subjects – BMI 25–29.9 kg/m² (2) and obese subjects – BMI >= 30 kg/m² (3)

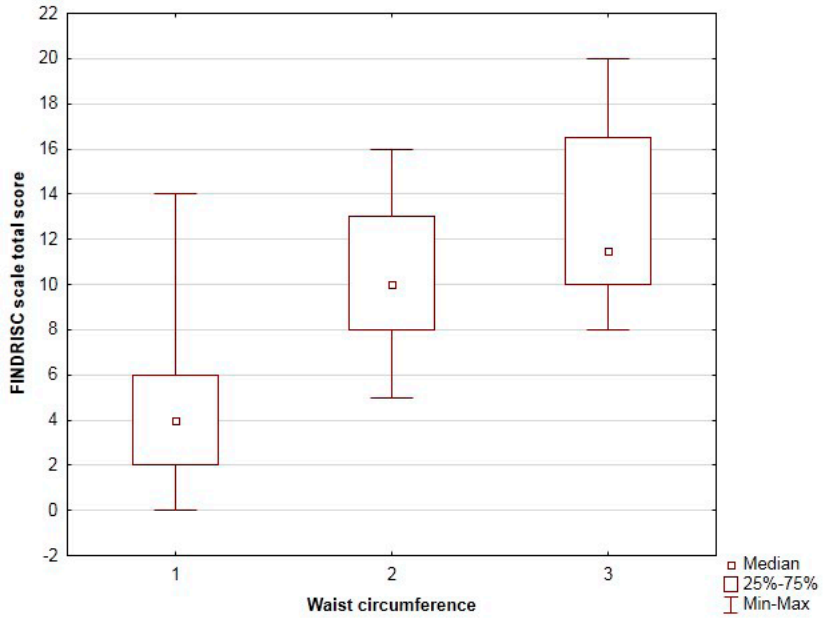

Figure 2. FINDRISC score in subjects with normal waist circumference – M < 94 cm, K < 80 cm (1), high waist circumference- M: 94–102 cm; F: 80–88 cm (2) and abdominal obesity – M > 102 cm; F > 88 cm (3)

antihypertensive drugs were excluded from the statistical analysis, systolic and diastolic blood pressure were found to be significantly higher in overweight or obese individuals than in those with normal body weight (the Kruskal-Wallis H test, $p < 0.05$) (**Figure 3**). However, no difference in blood pressure was noted between overweight and obese individuals.

Interestingly, a significant increase in systolic and diastolic blood pressure was noted in individuals with

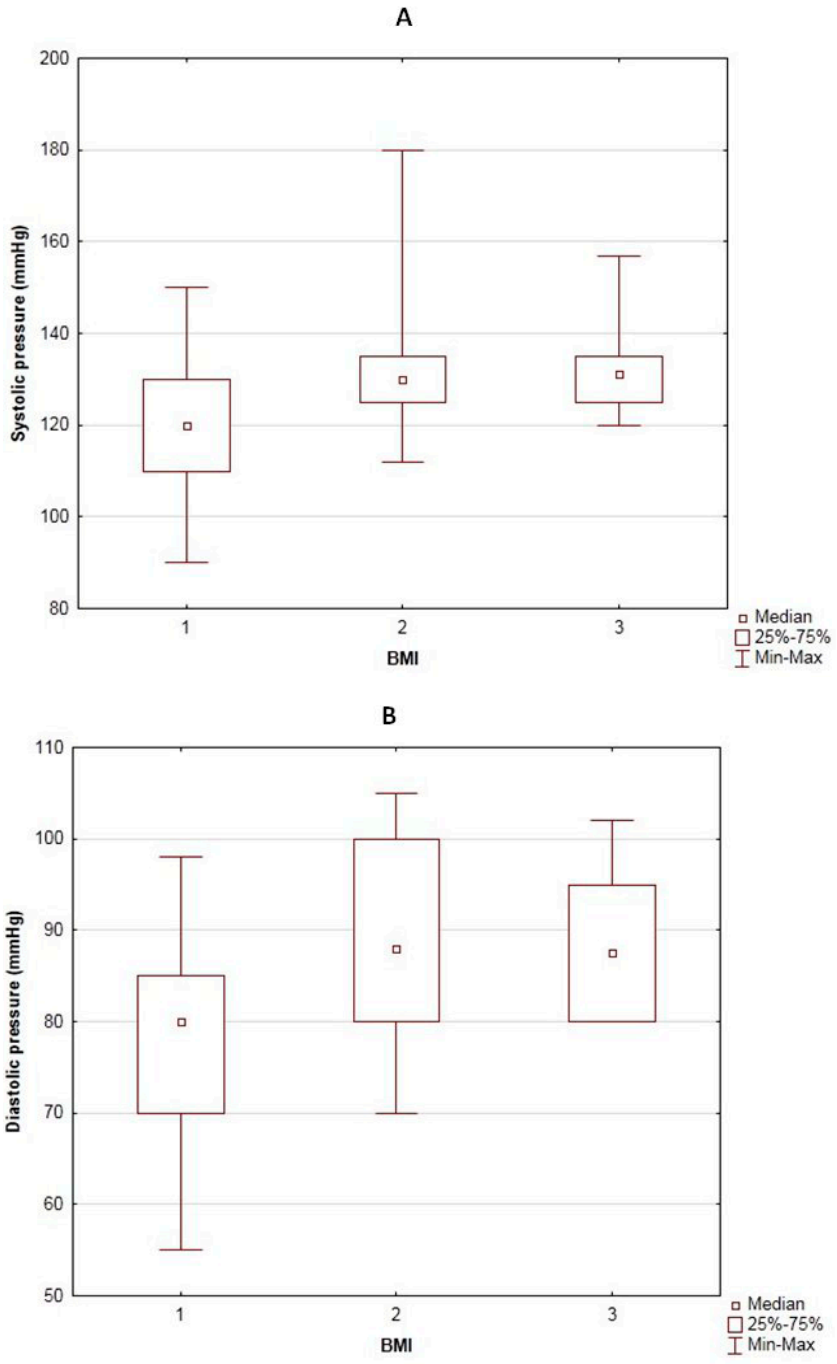

Figure 3. Systolic (A) and diastolic (B) blood pressure in subjects with normal BMI (1), overweight subjects (2) and obese subjects (3)

abdominal obesity (the Kruskal-Wallis H test, $p < 0.05$), but not in those with moderately high waist circumference (**Figure 4**).

Respondents with abdominal obesity reported a low level of physical activity statistically more frequently (the chi-squared test, $p < 0.05$), but no difference in physical activity was observed between slim individuals and those with elevated BMI. Obese individuals statistically more often reported eating less fruit and vegetables than those with normal body weight (the chi-squared test, $p < 0.05$).

Surprisingly, no differences were noted between individuals with normal body weight or waist circumference and those with excessive body weight in terms of history of hyperglycaemia or diabetes in the family.

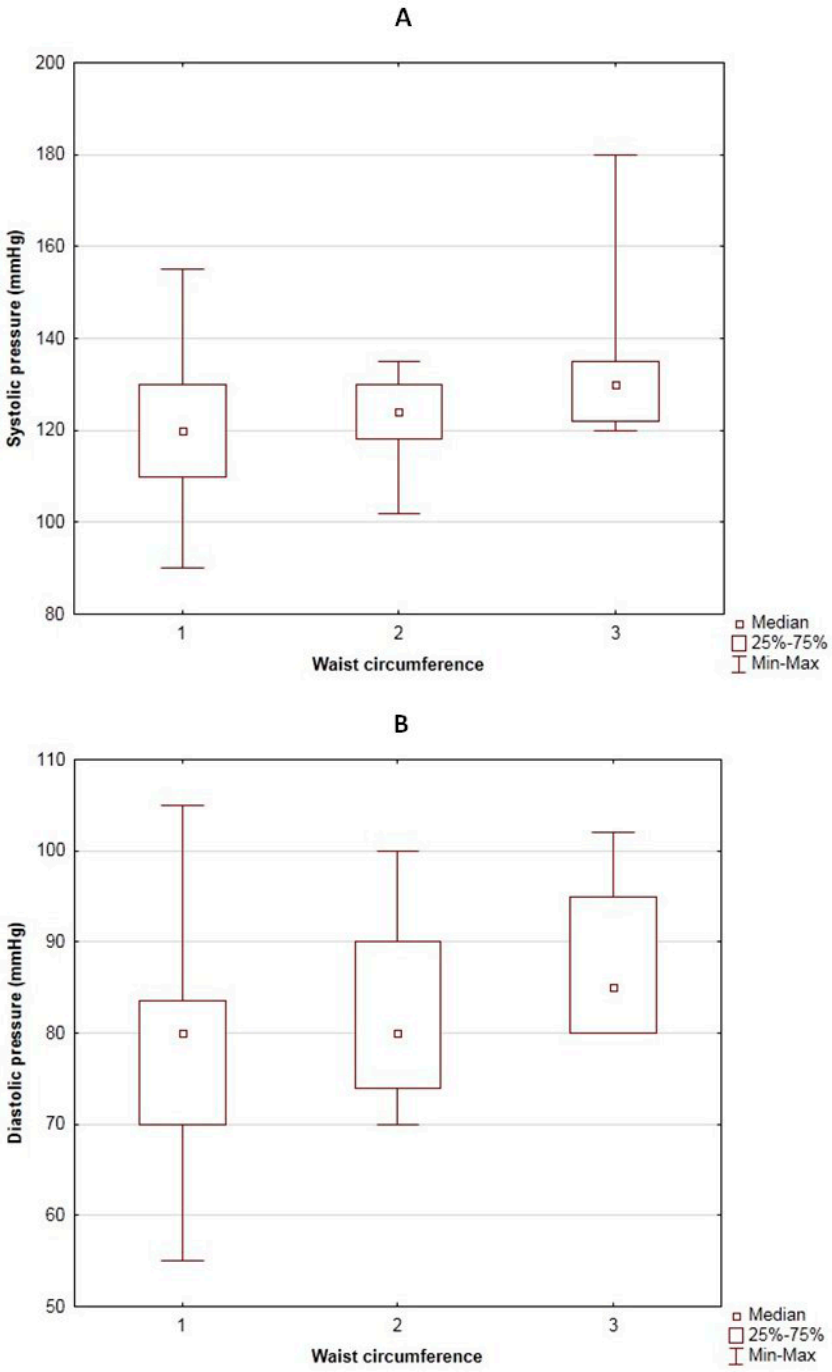

Figure 4. Systolic (A) and diastolic (B) blood pressure in subjects with normal waist circumference (1), high waist circumference (2) and abdominal obesity (3)

Discussion

People attending the 12th Lublin Science Festival were recruited for the study. Among these were relatively few individuals with BMI > 30 kg/m^2 (12 people). It seems likely that obese individuals preferred to stay at home [6] or chose not to participate in the study because they anticipated unfavourable results [7]. It has been demonstrated that obese individuals struggle in society with feelings of guilt or shame because of their weight, and are often unable to cope with these problems [8]. Patients with obesity have also frequently mental disorders [9]. This suggests the

need for a broad public initiative making it possible to reach these people and to take prophylactic and therapeutic measures to help them.

Our study shows that even individuals with a high FINDRISC score surprisingly seldom report a history of elevated blood glucose levels. One may speculate that these patients, were not regularly tested in the past and therefore they might have remained undiagnosed with hyperglycaemia. Government programmes to promote health and prevention by introducing regular obligatory glycaemia tests should play a greater role in preventing a further increase in the incidence of type 2 diabetes [10].

A family history of diabetes was also reported by few respondents. This could be explained both by a lack of knowledge of type 2 diabetes and by the growing problem of new cases of diabetes, or the increasing risk of this disease in individuals from previously healthy families [1].

The risk of diabetes and hypertension increases with BMI [11]. Interestingly, our results suggest that while high waist circumference raises the FINDRISC score, this does not continue to increase with a further increase in waist circumference. In contrast, increasing BMI is accompanied by a gradual increase in the FINDRISC score.

Our study also showed that obesity defined in terms of BMI did not fully correspond with obesity recognized on the basis of waist circumference. Other authors confirm these results, particularly in the case of women [12]. These data indicate that measurement of waist circumference is a somewhat better tool for identifying individuals with excess body weight than BMI.

Our study also showed that it is primarily waist circumference that increases with age, while BMI increases to a much lesser degree.

Another interesting observation is that BMI has a greater effect than waist circumference on blood pressure. This is confirmed by a study that showed that BMI is a more sensitive indicator of hypertension, whereas waist circumference is a better indicator of dyslipidaemia and diabetes [13]. Moreover, due to these differences WS Lee has recently proposed new algorithms defining the level of obesity in individuals [14].

Perspectives

High waist circumference raises the FINDRISC score, but the FINDRISC score does not continue to increase with waist circumference, whereas increasing BMI is accompanied by a gradual increase in the FINDRISC score. BMI has a stronger effect than waist circumference on blood pressure. General practitioners should encourage testing in patients and test all overweight and obese patients for development of type 2 diabetes. A public programme encouraging these individuals to undergo prophylactic examinations is necessary. On the other hand, nearly half of the respondents had normal BMI, which may be indicative of the growing awareness of the effect of an unhealthy lifestyle on the development of type 2 diabetes.

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Correspondence address:
Joanna Pekar
Students' Scientific Association at the Chair
and Department of Human Physiology
Medical University of Lublin,
11 Radziwillowska St, 20-080 Lublin, Poland
phone: +48783549595
email: asia9384@o2.pl

ORIGINAL PAPER

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The health locus of control in middle-aged low-risk patients qualified for coronary artery bypass grafting with extracorporeal circulation

Joanna Pielok[1], Włodzimierz Płotek[2], Regina Samborska[3], Marcin Cybulski[4]

[1] Operative and Post-Operative Department, Holy Family Hospital, Specialist Health Care Centre for Mother and Child, Poznań, Poland
[2] Department of Teaching Anaesthesiology and Intensive Therapy, Poznan University of Medical Sciences, Poland
[3] Department of Cardiac Surgery with Intensive Cardiological Supervision Wards, Józef Struś General City Hospital in Poznań, Poland
[4] Department of Clinical Psychology, Poznan University of Medical Sciences, Poland

ABSTRACT

Introduction. The health locus of control gives a possibility to determine the patient's self-efficacy resources, which are specific in locating health control actions. It also enables prediction of the type of health behaviours the patient will exhibit during recovery after a cardiac surgery.
Aim. The aim of the study was to use the Multidimensional Health Locus of Control (MHLC) to assess the occurrence of the internal health locus of control (IHLC), powerful others (PHLC) and chance (CHLC) in patients undergoing coronary artery bypass grafting according to their sex, occupational activity and education. The occurrence of types of health locus of control was also assessed.
Material and methods. 52 patients aged 47–63 years were tested (46 men – 88.5% and 6 women – 11.5%). The position of health control was tested by means of the Polish version of the American MHLC adapted by Juczyński.
Results. The average scores were as follows: I – 27.92 points, O – 29.60 points, C – 22.61 points. The research revealed statistical dependencies for some MHLC dimensions and for the sex and education. MHLC Type 7 – undifferentiated, strong (19 patients, 36.6%) was the most common.

Key words: anaesthesia, locus of control.

Introduction

Ischaemic heart disease is the most common disease of the cardiovascular system in developed countries. It turned out that somatic causes of the development of coronary artery disease and genetic conditions account for only 80% of its aetiology. The multi-centre research 'Interheart' resulted in identification of psychosocial factors as an independent cause of the development of ischaemic heart disease [1]. In daily medical practice it is difficult to make a precise psychological assessment of a patient scheduled for cardiac surgery because it requires broad knowledge and time. Therefore, we need research tools which will enable us to identify patients with high risk of occurrence of emotional and mental disorders, which will negatively affect the process of treatment and rehabilitation. The Multidimensional Health Locus of Control scale is one of such tools [2].

In the contemporary holistic model health is approached in a multidimensional manner. It consists of the physical, mental, spiritual and social dimension. In spite of the presence of a somatic disease many people remain healthy in the psychosocial

aspect or vice versa [3]. On the one hand, the occurrence of an illness results in passive, biological surrender to it. On the other hand, it results in a creative reaction to challenges, difficulties and threats brought by the illness. The effectiveness of reaction depends on the patient's perception of themselves in the situation of disordered balance between health and illness. Some people are convinced that they can control their response to the situation and have influence on harmful and negative events. Although these people need medical assistance, they actively participate in these events at all times. On the other hand, for other people an illness is an event which remains beyond their control. They are passive and assume that recovery is a result of external factors [4]. The relation between the patient's perception of their illness and their potential to cope with it and ability to exhibit health-promoting behaviour is defined as the health locus of control [5]. The construct of the health locus of control was based on the social learning theory developed by Rotter in 1954. According to the theory, one's own action is a tool to achieve the goal. Rotter distinguished between the internal and external locus of control. The sense of locus of control was defined as a causation between one's activity and the event which led to this activity. As far as the internal locus of control is concerned, events are the consequence of an individual's actions and personal control. The internal locus of control expresses one's efforts to control one's environment and emotions, to take responsibility for one's actions and take autonomous decisions. As far as the external locus of control is concerned, events are perceived to be determined by factors beyond one's personal control, independent of one's deliberate actions. People with the external locus of control think that they are guided by chance, fate and social environment. Everything in their lives depends on external factors, which are beyond their control [6].

Referring the theory of locus of control to health facilitates determination of one's attitude to illness and enables prediction of behavioural and cognitive actions during illness and recovery [7]. The internal health locus of control involves taking greater responsibility for one's health and it favours health. As far as the external health locus of control is concerned, the patient makes his/her recovery dependent on external factors, such as good luck, chance, belief or action of third parties. It is impossible to put the equals sign between chance and professional medical care. Therefore, the external health locus of control was divided into the one related to other people's influence and the one related to chance [8]. As far as the internal locus is concerned, health control depends on the patient. When we take other people's influence, health is the result of other people's actions, especially the result of actions taken by medical personnel [9]. The influence of chance means that health depends on random external factors. People with the internal health locus of control are characterised by greater optimism and actively solve problems. On the other hand, people with the external health locus of control react to difficult situations with greater stress and fear. The external health locus of control is positively correlated with neuroticism, whereas the internal health locus of control is negatively correlated with neuroticism [10].

There are differences in the health locus of control, which depend on respondents' age, sex, state of health and place of residence [11]. The internal health locus of control decreases with age, whereas the belief in other people's influence and chance increases with age. The tendency for the internal health locus of control is greater in men than in women [12]. This tendency is also greater in urban than in rural inhabitants. Studies comparing the health locus of control between healthy and sick people revealed that healthy people find the internal health locus of control more significant than sick people do. The lowest level of the internal health locus of control was observed in dialysed patients and in pregnant women, whereas other people's influence was rated highest by patients of oncological departments [13]. So far most publications have assessed and compared the health locus of control in healthy and chronically ill patients [14, 15]. There have been few observations concerning patients treated in hospitals, especially immediately before surgery [16, 17].

The aim of the study was to assess the types of health locus of control in patients qualified for coronary artery bypass grafting with extracorporeal circulation and to check the distribution of the MHLC types in the group under study, depending on the subjects' sex, education and occupational activity.

Material and methods

The research was planned according to the requirements of Good Clinical Practice included in the regulation issued by the Minister of Health on 10 December 2001 (based on Article 6, Paragraph 5, Section 5 of Pharmaceutical Law issued on 6 September 2001 – Official Journal No. 126, Pos. 1381 and Official

Journal 2001, No. 113, Section 984, No. 141, Section 1181 and No. 152, Section 1265).

On 1 March 2012 we received approval of the Bioethics Committee, Poznan University of Medical Sciences (Resolution No. 265/12). The research was conducted at the Department of Cardiac Surgery, Józef Struś General City Hospital in Poznań, Poland. We proposed participation in the research to patients living in the Poznań agglomeration (the fifth largest city in Poland) who were qualified and prepared for scheduled coronary artery bypass grafting with extracorporeal circulation. The patients were qualified for the surgery according to the current standards of the Department of Cardiac Surgery, Józef Struś General City Hospital in Poznań, which was in agreement with the generally accepted clinical practice. The patients met the inclusion criteria for the group under study.

The inclusion criteria were as follows:

- Patients scheduled for coronary artery bypass grafting under general anaesthesia with extracorporeal circulation;
- Ejection fraction before the surgery equal to or greater than 40%;
- Age 45–65 years;
- Native speakers of Polish
- At least eight years of primary school education
- Informed consent to participate in the research.

The exclusion criteria were as follows:

- Surgical emergencies;
- Surgeries with coronary artery bypass grafting and valve replacement, valve surgeries, aortic aneurysm surgeries or reoperations;
- Cerebrovascular accident (stroke, transient ischaemic attack) within 3 months before the surgery;
- Mental illness diagnosed and treated;
- Cognitive impairment: Mini Mental State Examination (MMSE) < 24 points, Schulman's Clock Drawing Test above the first level of errors, sense of coherence according to Antonovsky's subscale of reasonableness < 34 points;
- Permanent pacemaker;
- Chronic liver disease (understood as alanine aminotransferase (ALAT) and aspartate aminotransferase (AspAT) levels being twice as high as the norm in initial tests);
- Chronic renal failure diagnosed (creatinine level in initial tests > 2mg/dl);
- Chronic intake of psychotropic medications (understood as daily intake of these drugs for at least 3 months before the surgery);
- Alcoholism (understood as daily consumption of at least 25 g of pure alcohol or weekly consumption of 500 g of pure alcohol);
- Unregulated diabetes (understood as postprandial concentration of glucose above 11.1mmol/l and glycated haemoglobin of HbA1c > 9% [which was measured in patients with diagnosed diabetes and qualified for the research on the day before the surgery]);
- Preoperative anaemia (understood as haemoglobin (Hb) < 7.0mmol/l and haematocrit (HCT) < 34%);
- Hyperthyroidism or hypothyroidism
- No consent to the test.

72 patients were offered to take part in the research. 18 patients refused to participate for the following reasons: excessive preoperative anxiety (5 patients), unwillingness to take part in scientific research (7 patients), lack of glasses for reading (2 patients), inability to read (1 patient), no reason for refusal given (2 patients). One patient was not qualified for the research due to incorrect results obtained in screening tests (MMSE <24 points; Clock Drawing Test –the fourth level of errors). One patient was disqualified because he admitted his wife had done the test for him.

Research tools

Multidimensional Health Locus of Control (MHLC) version B

The Polish version of the American Multidimensional Health Locus of Control (MHLC) scale was adapted by Juczyński. It enables identification of generalised expectations in three dimensions of health control: the internal health locus of control (IHLC), the powerful others health locus of control (PHLC), and the chance health locus of control (CHLC). The scale is a self-report tool. It contains 18 statements about the health locus of control. The respondent is supposed to rate them using a six-point scale provided above the statements. Among the 18 statements in the scale, 6 statements concern the IHLC, 6 statements concern the PHLC, and 6 statements concern the CHLC. The minimum score for each scale is 6 points, whereas the maximum score is 36 points. The higher the score is, the stronger the respondent's belief is that this factor has influence on their state of health. Depending on the interrelation between the three dimensions, the score is allocated to one of eight MHLC types, according to demographic standards. The internal consistency (Cronbach's alpha) is 0.74 for I, 0.69 for O and 0.54 for C [18].

Research procedure

The patients who agreed to take part in the research and met the inclusion criteria were allocated to one of three groups, depending on their sex, occupational activity and education. One day before the surgery the patients were requested to respond to the questions provided in the MHLC test. When the scores were allocated to one of the types of health locus of control, the dependence between the types and the respondents' sex, occupational activity and education was analysed.

Statistical analysis

The statistical analysis was made with a computer package for statistical calculations SPSS v.21.

When describing basic biometric data, MHLC results and cardiac surgery data were presented as minimum, maximum and mean values as well as standard deviation. When describing the distribution of data in the subgroups of sex, occupational activity and education, standard error of the mean was also added. The results of the patients qualified for IHLC, PHLC and CHLC in the MHLC test were correlated with their sex, occupational activity and education. When analysing sex-dependent and occupational activity-dependent differences between the subgroups in individual MHLC dimensions, the homogeneity of variance was checked with Levene's test. Next, Student's t-test was conducted. ANOVA and post-hoc LSD test were used to assess the influence of education on the results. In all statistical tests $p < 0.05$ was assumed as the limit of statistical significance.

Results

Research group characteristics

The research was completed by 52 patients aged 47–63 years (middle adulthood according to Erikson) [19]. 46 men (88.5%) and 6 women (11.5%) took part in the research. 6 patients (11.5%) had primary education, 24 patients (46.2%) – vocational education, 14 patients (26.9%) – secondary education, 8 patients (15.4%) – higher education. 31 patients (59.6%) were employed and 21 patients (40.4%) were unemployed. None of the unemployed patients received a disability pension due to cardiac diseases. The patients' biometric data and ejection fraction are shown in **Table 1**.

Concomitant diseases

The most common concomitant diseases were:

- hypertension – 24 patients (46.2%);
- nicotine addiction – 14 patients (26.9%);
- myocardial infarction – 11 patients (21.2%);
- diabetes with stabilised blood glucose – 5 patients (9.6%);
- gout – 5 patients (9.6%);
- bronchial asthma, chronic obstructive pulmonary disease, active stomach ulcers, hypothyroidism treated by a specialist endocrinologist during euthyroidism, obliterating arteritis – 2 patients (3.8%) with each disease;
- prostate cancer after hormonal treatment in remission, rheumatoid arthritis, systemic lupus erythematosus, psoriasis - without treatment, nasal polyps - 1 patient (1.9%) with each disease.

MHLC dimensions

- The mean scores in the research group were as follows: IHLC: 27.92 points (SD – 5.19), PHLC: 29.60 points (SD – 4.08), CHLC: 22.61 points (SD – 6.08). Detailed data can be found in **Table 2**.
- The mean scores – according to the respondents' sex, occupational activity and education

  The mean value of the scores in individual dimensions was compared in relation to the respondents' sex. Detailed data can be found in **Table 3**.

The analysis revealed a statistically significant difference only in dimension PHLC, where the men had higher scores. However, the results were close to the limit of statistical significance (Student's t-test, $p = 0.047$).

The analysis of the scores in the MHLC dimensions in different groups of occupational activity did not show statistically significant differences despite differences between the scores (Student's t-test, $p > 0.05$). Detailed data can be found in **Table 4**.

Table 1. The research participants' body weight, height, body mass index, body surface area and ejection fraction

Basic biometric parameters	N	Minimum	Maximum	Medium	Standard deviation
Body weight (kg)	52	54.00	143.00	86.28	17.76
Height (cm)	52	156.00	187.00	172.13	7.51
Body Mass Index (kg/m^2)	52	21.60	46.20	28.91	4.6
Body Surface Area (m^2)	52	1.59	2.51	1.20	0.21
Ejection Fraction (EF) (%)	52	40.00	61.00	51.19	5.88

Table 2. Descriptive statistics of the MHLC test results in the whole group (N = 52)

Dimensions of the MHLC	Minimum	Maximum	Mean	Standard deviation
IHLC	14.00	36.00	27.92	5.19
PHLC	12.00	36.00	29.60	4.08
CHLC	10.00	35.00	22.61	6.08

MHLC - Multidimensional Health Locus of Control scale; IHLC – Internal Locus of Control, PHLC- Powerful Others Locus of Control, CHLC – Influence of Chance Locus of Control

Table 3. A comparison of mean scores in the MHLC scale according to the participants' sex

Dimensions of the MHLC	Sex	N	Mean	Standard deviation	Standard error of mean
IHLC	men	46	28.30	4.96	0.73
	women	6	25.00	6.48	2.65
PHLC	men	46	30.00*	3.40	0.50
	women	6	26.50*	7.29	2.97
CHLC	men	46	22.09	5.98	0.89
	women	6	26.67	5.78	2.36

* - statistically significant
MHLC - Multidimensional Health Locus of Control scale; IHLC – Internal Locus of Control, PHLC – Powerful Others Locus of Control, CHLC – Chance Locus of Control

Table 4. A comparison of mean scores in the MHLC scale according to the participants' occupational activity

Dimensions of the MHLC	Occupational activity	N	Mean	Standard deviation	Standard error of mean
IHLC	employed	31	28.19	4.76	0.86
	unemployed	21	27.52	5.87	1.28
PHLC	employed	31	29.74	3.38	0.61
	unemployed	21	29.38	5.02	1.10
CHLC	employed	31	21.55	5.92	1.06
	unemployed	21	24.19	6.11	1.33

MHLC - Multidimensional Health Locus of Control scale; IHLC – Internal Locus of Control, PHLC – Powerful Others Locus of Control, CHLC – Chance Locus of Control

Table 5. A comparison of mean scores in the MHLC scale according to the participants' education

Dimensions of the MHLC	Education	N	Mean	Standard deviation	Standard error of mean
IHLC	primary	6	30.83	4.21	1.72
	vocational	24	28.46	4.05	0.83
	secondary	14	26.57	6.65	1.78
	higher	8	26.50	5.78	2.04
PHLC	primary	6	30.67*	2.34	0.95
	vocational	24	30.46*	3.36	0.67
	secondary	14	29.00	5.45	1.46
	higher	8	27.25*	4.08	0.57
CHLC	primary	6	25.83	6.55	2.68
	vocational	24	24.25	5.19	1.06
	secondary	14	20.71	6.60	1.76
	higher	8	18.63	5.07	1.79

* - statistically significant
MHLC - Multidimensional Health Locus of Control scale: IHLC – Internal Locus of Control, PHLC – Powerful Others Locus of Control, CHLC – Chance Locus of Control

The mean value of the scores in individual dimensions differed according to the respondents' education. Detailed data can be found in **Table 5**.

The ANOVA test did not reveal statistically significant differences in IHLC or PHLC. As far as CHLC is concerned, the mean values in the groups with primary and vocational education were greater and they were significantly different from the mean value in the group with higher education (ANOVA F = 3.077, p = 0.036). The post-hoc LSD test resulted in p = 0.024 for dif-

Table 6. The occurrence of MHLC types according to the participants' sex, education and occupational activity

			MHLC Type						Total
			2	3	4	5	6	7	
Sex	women	number	1		1	1	1	2	6
		%MHLC	16.67		16.67		16.67	33.33	100
	men	number	3	2	11	14		17	46
		%MHLC	6.52	2.17	23.91	30.44		36.96	100
Occupational activity	unemployed	number	2		5	5	1	8	21
		%MHLC	9.52		23.81	23.81	4.76	38.10	100
	employed	number	2	1	8	9		11	
		%MHLC	6.45	3.22	25.81	29.04		35.48	100
Education	primary	number			1			5	6
		%MHLC			16.67			83.33	100
	vocational	number	5	1	2	7		9	24
		%MHLC	20.83	4.17	8.33	29.17		37.50	100
	secondary	number			4	5	1	4	14
		%MHLC			28.57	35.71	7.15	28.57	100
	higher	number			4	3		1	8
		%MHLC			50	37.5		12.5	100

MHLC - Multidimensional Health Locus of Control Scale

ferences between higher and primary education and p = 0.02 for differences between higher and vocational education.

MHLC types

Next, according to the test methodology, the patients were qualified for one of the eight types of health locus of control. The most common was type 7: undifferentiated strong – it was observed in 19 respondents (36.6%).

The frequency of occurrence of individual MHLC types was checked according to the respondents' sex, occupational activity and education.

Type 7 was the most common among the women (33.33%). Among the men the following three MHLC types were predominant: type 7 (36.96%), type 5 (23.91%) and type 4 (23.91%).

The distribution of the types of health locus of control in the subgroups of employed and unemployed patients was similar. Type 7 was the most common in both subgroups – it was found in 38.10% of unemployed patients and in 35.48% of employed respondents.

The patients with primary education exhibited only two types of health locus of control. Most of them (83.33%) were qualified for type 7. The patients with vocational education exhibited five MHLC types, but type 7 (37.5%) and type 5 (29.17%) were the most common. Type 5 was the most common among the patients with secondary education (35.71%). In the group of patients with higher education type 4 was the most common (37.5%). In contrast to the other groups, type 7 was the least common in this group. Detailed data can be found in **Table 6**. Due to the small number of research participants in individual MHLC types the data were only used for observation and no detailed statistical calculations were made.

Discussion

The Multidimensional Health Locus of Control (MHLC) was developed by Wallston et al. It enables assessment of individual competences in developing one's behaviour in health and illness. The scale illustrates three dimensions of health locus of control: the internal, the powerful others and influence of chance. On the one hand, the identification of the type of health locus of control in patients undergoing surgeries gives a possibility to determine how an individual can cope with stressful situations. On the other hand, it enables prediction how the patient's immunity resources may influence the course of postoperative therapy [20]. The health locus of control depends on respondents' age, their place of residence, state of health and socioeconomic conditions. In this study the participants were residents of an urban agglomeration, aged 47–63 years (middle adulthood). They had a short medical history of ischaemic heart disease, which did not limit their current life activity. Having conducted screening tests, those participants were included in the research who did not suffer from concomitant cognitive disorder or depression.

Laboratory tests confirmed that the research participants were in good somatic condition. They did not suffer from significant concomitant diseases which might affect their health locus of control. Before the research we assumed that the variables which might affect the respondents' health locus of control were their sex, education and occupational activity. The analysis of the results revealed that they were similar in all the three dimensions. The participants' scores were the highest in PHLC and slightly lower in dimension I. The CHLC had the lowest scores. In one of few studies on patients who underwent coronary artery bypass grafting Sorlie observed that in comparison with the general surgery group they were characterised by higher internal health locus of control. It resulted from three factors. First of all, ischaemic heart disease affected the patient's lifestyle. Second of all, these patients contacted cardiologists more often. Third of all, the qualification and preparation for the surgery were more standardised than in other branches of surgery. According to Sorlie, due to these three factors patients had better knowledge of their illness and they knew and followed the rules of health-promoting behaviour [16]. In our study the results were different because the patients rated other people's influence higher than their internal control. This situation may have resulted from the following three reasons. First of all, most of the patients in the research group had a short medical history and during the preoperative period none of them exhibited the symptoms which would force them to change their lifestyle. Apart from that, health education in Poland is less developed than in Scandinavia and patients' contact with the cardiologist is limited. The third factor of equal importance which may have affected the lower internal health locus of control in our group of patients was different economic status. According to Fitzgerald, the higher the economic status and everyday living standard are, the stronger the internal health locus of control is [21]. However, when we compare our findings with the results received from patients in cardiac centres in Poland, they are very similar. Guzińska conducted research on patients participating in the rehabilitation treatment after coronary artery bypass grafting surgeries. In the first test, which was conducted at the beginning of the rehabilitation treatment, the results were similar to ours. The highest score was noted for PHLC, average score for IHLC and the lowest score for the CHLC. When the test was repeated after the rehabilitation treatment, when the patients had been instructed by experts how to live with their illness, IHLC was rated higher, whereas PHLC decreased [22]. Opuchlik studied a group of 60 patients with ischaemic heart disease and hypertension. Like in our study, the patients' scores were the highest in PHLC and the lowest in CHLC [23]. Kurowska studied 97 patients with hypertension. She found that the patients' scores were the highest in IHLC, average in PHLC and lowest in CHLC [24]. In Kurowska's study men had higher scores than women in all of the three dimensions. In Opuchlik's study, like in ours, men's scores were higher in IHLC and PHLC, whereas women's scores were higher in CHLC [23, 24]. In our study there were many more men than women. It limits the interpretation of data, but it seems inevitable because ischaemic heart disease and coronary artery bypass grafting surgeries are more common in men. In our study there was a statistically significant difference between men's and women's scores in PHLC. However, in view of the fact that the statistical analysis produced the result close to the limit, it should be interpreted with due care because of high disproportion between the subgroups of men and women. The result may have been coincidental. Further, more detailed research might result in more definite conclusions.

There were also differences in the results, depending on the respondents' occupational activity. In spite of the fact that the statistical analysis did not reveal significant differences, the employed respondents' mean scores were slightly higher in the IHLC and in the dimension of PHLC, whereas the unemployed respondents' mean scores were slightly higher in the CHLC. This observation is in agreement with most other studies, which indicate that unemployed people are characterised by much higher external health locus of control than employed respondents [21, 25].

In our study the respondents' scores also differed depending on their education. The comparison of IHLC, PHLC and CHLC between the groups of education revealed that the patients with primary education had the highest score in PHLC. It is noteworthy that as the respondents' education grew higher, the mean value of CHLC decreased, resulting in a statistically significant difference between the patients with higher education and those with primary or vocational education. Most studies assessing the health locus of control according to socioeconomic conditions show that as the level of education increases, it is positively correlated with the internal health locus of control, whereas the external health locus of control is negatively correlated with education [26, 27]. However, some reports negate

this dependence [26]. Most studies assessing the relation between education and health locus of control are conducted on a very large number of people. This population is usually very diversified and includes both healthy subjects and those suffering from different, often chronic illnesses, whose influence was not taken into consideration in the assessment of health locus of control [26, 28]. In our study there were selected cardiac surgery patients without significant concomitant diseases. As Sorlie reports, this group of patients is characterised by greater internal health locus of control than the rest of the population [16]. It cannot be ruled out that the construction of the MHLC test itself caused such high scores in the group with primary education. The result may have depended on the possibility to gain introspective insight. When the patients were responding to the questions, they had to choose one of the descriptive statements in the test. When they chose extreme responses, i.e. "Strongly agree" or "Strongly disagree" they had no doubt about the right response. The choice of less definite responses was more difficult. It resulted from the fact that there is a relatively subtle difference between the statements "Slightly agree" and "Slightly disagree" and it requires longer consideration. For some patients, especially those with primary or vocational education, this difference was indistinguishable and therefore, they tended to give more extreme and definite responses.

In our study the greatest number of patients exhibited the undifferentiated, strong type (36%), followed by the type lessening the influence of chance (28.8%). 23% of the patients maximised the influence of chance, whereas 7.7% of the patients belonged to the strong external type. The type lessening other people's influence and the type maximising the influence of chance was observed only in 1.9% of the patients. None of the research participants was qualified to either of the extreme types of health locus of control, i.e. type 1 – strong, internal, or type 8 – undifferentiated, weak. None of the participants exhibited the undifferentiated, weak type, whereas the undifferentiated, strong type was the most common. In the only available study analysing the occurrence of MHLC types in cardiac patients the largest group was characterised by the undifferentiated, weak type, whereas the smallest number exhibited the undifferentiated, strong type and the type maximising other people's influence [24]. By contrast, in our study the patients were a more homogenous group in terms of their age, concomitant diseases and history of their illness. This may have caused different distribution of MHLC types in the groups under study. Sex and occupational activity did not influence the frequency of occurrence of MHLC types. Type 7 – undifferentiated, strong was the most common both among the men and women, regardless of their employment or unemployment. The analysis of dependence between the types of health locus of control and education revealed that as the patients' level of education increased, so did the diversity of types exhibited and there was variation in the most common MHLC type. Type 7 was predominant among the respondents with primary or vocational education, type 5 – among the respondents with secondary education and type 4 – among 50% of the respondents with higher education. It is also noteworthy that type 7 was the least common in this group of education. Unfortunately, we have not found a study with the results that could be compared with our observations.

In view of the investigations which have been conducted so far, it seems that the identification of the type of health locus of control in patients qualified for cardiac surgeries might help to individualise postoperative treatment and further rehabilitation [20, 22]. Patients with the internal health locus of control try to improve and maintain their state of health and they use social support effectively [29]. By contrast, patients with the external health locus of control, which is dependent on other people's influence, tend to be more passive and follow other people's decisions [30]. According to Luszczynska, on the one hand, due to passiveness, patients consume less alcohol, smoke less and eat more fruit and vegetables. On the other hand, they make healthy physical effort less frequently, do not clean their teeth so often and consume more salt [31]. According to the study by Bergvik, the patients whose health locus of control depends on other people see doctors less often and start appropriate therapy later [32]. They are also characterised by neuroticism, which results in their greater tendency to react to stressful situations with fear and negative emotions [23, 31]. On the contrary, Kurowska arrived at different conclusions. She claims that patients with the external health locus of control are characterised by better health-promoting behaviours and greater optimism. In consequence, they pay more attention to health-promoting practices than patients with the internal health locus of control [24]. Unlike Kurowska, Kugler observed in his study that in the group of patients awaiting heart transplantation the external health locus of control, i.e. both PHLC and CHLC, was related with high preoperative fear and depression. Divergent observations made by different authors point to the need to continue research on

the subject. Also, during the postoperative period fear and depression were more often observed in patients with the external health locus of control [33]. Reynaert made interesting observations on patients undergoing scheduled cardiac surgeries. Patients with the internal health locus of control reported lesser intensity of postoperative pain than those with the external health locus of control. During the study it turned out that patients with the internal health locus of control consumed 40% less morphine in patient-controlled analgesia than other patients [34]. The Norwegian study also shows that the type of health locus of control is related with patients' life activity after coronary artery bypass grafting and percutaneous transluminal coronary angioplasty. The study was conducted on a large group of 348 patients and revealed that the occurrence of the internal health locus of control was positively correlated with returning to work and life activity before the illness. The patients whose type of health locus of control depended on other people less frequently returned to their occupational activity after the therapy [32]. Burker analysed the fate of 100 patients who underwent lung transplantation. It turned out that the patients with a high or even medium internal health locus of control were characterised by longer survival rate than the patients with a low internal health locus of control [35].

Due to the fact that the health locus of control identifies patients' individual competence in the form of their own efficacy in the location of control of activities related with health, it gives a possibility to predict whether the patients will take responsibility for their health in the long-lasting process of cardiac surgery treatment. It also enables assessment of the patient's ability to cope with different stressful situations. It seems to be a valuable tool for identifying a group of patients who need help to control their fear and negative emotions related with a scheduled cardiac surgery. Identification of the type of health locus of control gives a possibility not only to choose a group of patients in need of psychological assistance but also to select appropriate instruments for effective behavioural therapy [5, 20, 29]. As it has turned out, in spite of the fact that the health locus of control is a relatively stable construct, an appropriate cognitive therapy may strengthen patients' internal health locus of control and thus, improve the effects of the long-lasting process of treatment [36]. Therefore, it seems that hospitalised patients may benefit from a broad-spectrum psychological examination during the perioperative period. The examination is not only a diagnosis, but it may also help to implement the therapeutic procedure. In view of this fact, the popularisation of knowledge of MHLC may strengthen the holistic approach of medical staff to patients.

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Correspondence address:
Włodzimierz Płotek
Department of Teaching Anaesthesiology
and Intensive Therapy
Poznan University of Medical Sciences
14 Św. Marii Magdaleny St, 61-861 Poznań, Poland
phone: +48616687836
email: plotekw@poczta.onet.pl

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Sympathetic skin response following single and combined sound and electrical stimuli in young healthy subjects

Agnieszka Wiertel-Krawczuk, Adam S. Hirschfeld, Juliusz Huber, Magdalena Wojtysiak, Agnieszka Szymankiewicz-Szukała

Department of Pathophysiology of Locomotor Organs, Poznan University of Medical Sciences, Poland

ABSTRACT

Introduction. Sympathetic skin response (SSR) is applied in evaluation of dysfunctions in autonomic nervous system. Among others, electrical and sound stimuli are most frequently used to evoke SSR.
Aim. The aim of this study was to determine if the bell ring stimulus with parameters different from standard sound stimulation evokes similar reactions in autonomic system as electrical stimulus with defined parameters.
Material and methods. SSR parameters were recorded following simultaneous sound and electrical stimulation. Twenty young volunteers (aged 23 ± 2.1 years) were examined once with SSR and R-R interval variation (RRIV) tests in order to confirm lack of functional changes in autonomic nervous system.
Results. Values of mean amplitudes of SSR were always higher during recordings from upper limbs than the lower ones irrespective of the three types of applied stimuli. Mean values of latencies were comparable when SSR were induced with acoustic, electrical and both stimuli during recordings performed from upper and lower extremities. Bell ring stimulus influenced only mean values of SSR area recorded both from upper ($p \leqslant 0.011$) and lower ($p \leqslant 0.023$) extremities. Heart beats variability in RRIV recordings changed at 13.5% which is comparable to results obtained by other authors.
Conclusions. Results indicate that the application of different modalities stimuli evokes SSR with comparable parameters. Each of them can be used for objective evaluation of the sympathetic nervous system function. Both SSR and RRIV tests evaluating the function of two effector types should be applied for the diagnosis of the probable dysautonomia in patients who show unclear clinical symptoms.

Keywords: sympathetic skin response, acoustic stimulus, electrical stimulus, R-R interval variation, normative values, autonomic system.

Introduction

The clinical evaluation of autonomic system disorders can be difficult because of the unspecific symptoms [1]. Ascertaining of dysautonomia using accurate and non-invasive methods of functional diagnostic is crucial [2]. The results of sympathetic skin response – SSR and the analysis of heart rate variability – RRIV functional examinations constitute the completion of its clinical diagnosis [3, 4]. Among the many tests to assess the function of the autonomic nervous system, the above-mentioned two types of tests are the most commonly used to confirm dysautonomia. The reaction of two kinds of effectors following the autonomic nervous system activation allows evaluating its function precisely.

Examination of the sympathetic skin response is based on the temporary changes in the electrical skin resistance caused by the synchronous activation of the

sweat glands. Applications of an unexpected external stimulus such as electrical (stimulation of median, tibial, peroneal or supraorbital nerves), physiological (sound, flash or touch) as well as emotional excitation evoke SSR [5–8]. Usually the electrical stimulus with 0.2ms duration and 15–30mA intensity is applied or the acoustic stimulus with the frequency of 100Hz, 100ms duration and 80 dB intensity via earphone biaural stimulation [9]. Considering the fact that sweat gland excitation can be the result from stimuli of different modality (e.g. emotional), the assessment of possible SSR parameters variability is interesting. Changes of SSR latency and amplitude are also not fully explained in the context of the application of the two stimuli simultaneously [8, 10], together with including the bell ring stimulation with different parameters than commonly accepted.

The heart rate variability depends on the phase and depth of breathing and it is controlled by vagal cholinergic impulsation which acting on the sino-atrial node slows the heart beats. During inspiration the parasympathetic activation is reduced. The opposite situation occurs in the expiratory phase. Deep breathing leads to changes in the autonomic system activity, thus increasing the heart rate variability [1, 2].

Aim

The aim of this study is to evaluate possible changes in SSR parameters by ascertaining their reference values in young, healthy volunteers aged from 19 to 27, when two types of stimuli such us bell ring (with parameters different than those applied in bi-aural with headphones) and electrical are used separately or simultaneously. The hypothesis has been put forward that every type of stimuli evokes SSR with the similar, repeatable parameters. Autonomic R-R interval variation (RRIV) examination is used in this study to confirm no pathology in cardiovagal function of the subjects.

Material and Methods

Subjects

The project was carried out from March to September, 2014. The final group included twenty young healthy volunteers who were examined once. There were 10 women aged from 22 to 27 (24 ± 2 years on average), 1.67 ± 0.09 m in height and 10 men aged from 19 to 26 (22 ± 2 years on average) 1.8 ± 0.06 m in height. The inclusion criteria were as follows: young subjects, who presented a general good health condition, having similar anthropometric properties, reported no neurological disorders, no autonomic diseases basing on the medical history from general practitioner. All subjects denied previous alcohol or drugs abuse. All participants gave written informed consent prior to the study. Ethical considerations were in agreement with the Helsinki Declaration. Demographical data of the examined subjects (N = 20) are presented in **Table 1.**

Instruments

The subjects were examined in a supine position in an air-conditioned room where the temperature was kept at 23°C on average. They were given 15 minutes of relaxation prior to the applied tests. The regime of quiet was rigorously kept. The same team of investigators performed all examinations. Four-channel KeyPoint system (Medtronic A/S Skovlunde Denmark) was used for recordings. The upper filter was set on 2 kHz, the lower one on 0.5 Hz. During recordings of SSR and RRIV the time base was set on 1 s/D and 200 ms/D and amplifications from 0.5 to 1 mV, respectively. RRIV was recorded for 60 s.

Surface bipolar stimulating electrode was placed over the right median nerve unilaterally at the wrist. Rectangular electrical "train" stimuli at the intensity of 10 mA, duration of 0.1 ms and frequency at 3 Hz in SSR studies were used (**Figure 1**). The impendency between the skin and the surface electrode did not exceed 5 kΩ. In order to obtain the lowest impendency, the skin was cleared with special paste which did not contain alcohol as the possible factor influencing the secretion of sweat glands. The surface AgCl adhesive disposable electrodes (recording surface 7 x 4 mm) were covered with a small amount of the isotonic gel to diminish the resistance between electrode plates and the skin. The active recording electrodes were placed in the middle of palms and soles at the areas between 2nd and 3rd metacarpus and metatarsus, respectively. Reference electrodes were placed centrally on the dorsal aspects of hands and feet. Ground electrodes were placed unilaterally at the wrist and ankle proximately

Table 1. Demographic data on examined subjects (mean values±SD)

Control group (N = 20)	Age (years)	Height (m)	Weight (kg)	BMI
♂ = 10 ♀ = 10	23 ± 2.1	1.74 ± 0.1	64.5 ± 10.4	21.28 ± 1.7

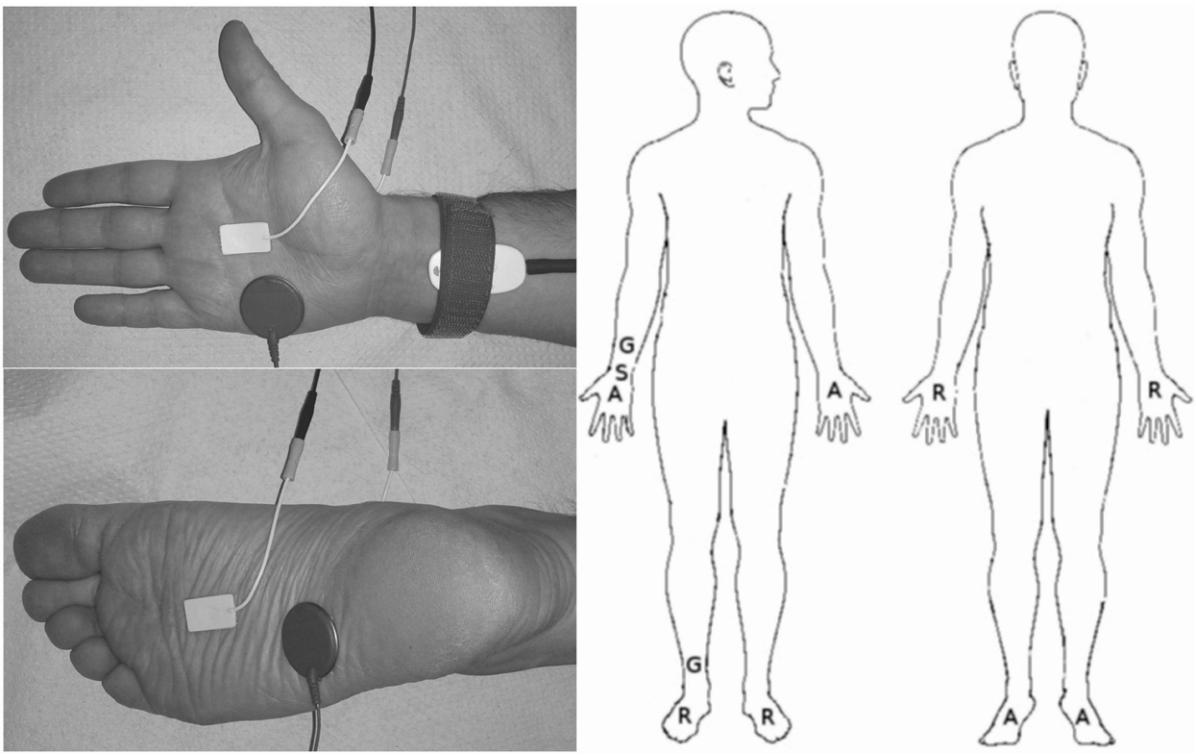

Figure 1. Scheme of the electrodes locations used in SSR studies. S – stimulating electrode, A –active recording electrode, R – reference recording electrode, G –ground electrode

to the recording electrodes according to descriptions of Claus and Schondorf [6] and Lee and DeLisa [11].

Three attempts for each session of SSR recordings from upper and lower extremities were conducted. In order to avoid the possible habituation, the irregular interval between each trial was applied. Latencies, amplitudes, areas and configuration (number of phases) of SSR were analysed. The latency was ascertained from the stimulus artefact following the first negative deflexion from baseline, it means to the onset of potential (in seconds). The amplitude was measured from the peak of the negative to the positive component (in millivolts).

The bell ring stimulus was also used to evoke SSR. No headphones were used. Every time the sound stimulus was applied from the same distance of the subject's head (approximately at 30 cm) and, which is important, stimulus application was simultaneously triggered with the onset of the recording indicated by marker. This eliminated the possible delay in reaction of sweat glands to stimulus, which might have determined the recordings latency variability. The frequency was kept at 3–4 Hz with the sound maximal intensity at 85 dB (64 dB on average). The acoustic stimulus was delivered for 3 s. The third way of evoking SSR was a combined bell-electrical stimulus induced in the same way as the one described separately. The bell ring and electrical stimuli were delivered exactly at the same moment and they triggered the onset of recording.

SSR responses with the shortest latencies were analysed. If the repeated stimulation brought no responses, the difference in latencies of recorded responses on both sides was greater than 50% or the results exceeded mean ± 2 SD values they were not included in the final analysis (N = 4).

The analysis of the R-R interval variation was performed with an active electrode placed on the 4th left intercostal space in the parasternal line and the reference electrode was placed in the right first intercostal space of the mammillary line. The ground electrode was located on sternum [12]. RRIV analysis included one minute recording during the normal (individual rhythm) and deep, regular breath (six breaths per minute) [13]. The percentage of changes in variability of R-R intervals then was assessed, as well as the frequency of heart beats per minute on both stages of examination. The improper result was ascertained as exceeding mean ± 2 SD values or the characteristic invariable (homogenic) recording was observed.

Statistical analysis

SSR parameters were described as ranges, mean values and their standard deviations. RRIV measure-

ments results were expressed as mean percentages of changes in R-R intervals basing on equation:

$$\% = \frac{\text{max.value} - \text{min.value}}{\text{mean value}}$$

The normality distribution test for SSR parameters was performed with Shapiro-Wilk test. The homogeneity of variances recorded on both sides and both sexes was tested with Mann-Whitney test. Differences in parameters of SSR induced with three types of stimuli were verified with ANOVA Friedmann test. The level of statistical significance was accepted at $p \leqslant 0.05$. All statistical analyses were performed using Statistica PL software version 9.0 (by StatSoft).

Results

During preliminary analysis the statistically significant differences at $p < 0.05$ between parameters of SSR recordings performed on both sides were not found, hence the results obtained in 20 volunteers were considered together as N = 40. The data regarding the obtained parameters in SSR recordings evoked with three types of stimuli are presented in **Table 2**.

Examples of SSR recordings are presented in **Figure 2**. Mean values of SSR amplitudes (mainly biphasic, with the first negative inflection) were always higher when recorded from the upper extremities, than from the lower ones, irrespective of the type of the applied stimulus. The mean values of latencies were also comparable when recorded from upper and lower extremities. It means the type of stimulus not change their values (**Table 2**).

The results of Friedmann's test presented in **Table 3** show that the type of stimulus influenced only mean values of the SSR area parameter recorded both in upper ($p \leqslant 0.011$) and lower ($p \leqslant 0.023$) extremities.

The relation between amplitude and area parameters can be clearly observed in histograms shown in **Figure 3**. The SSR area parameter was significantly smaller when the bell ring stimulus was applied than with the application of electrical and electrical-sound combined stimuli. The reduction of SSR area was caused by lower value of its amplitude during bell ring

Table 2. Data on results from SSR examination during recordings from upper and lower extremities following all three applied types of stimuli. Ranges, means and standard deviations are shown

Parameters	Upper extremities recordings (N = 40)	Lower extremities recordings (N = 40)
	Electrical stimulation	
Amplitude (mV)	0.19–2.36 1.13 ± 0.58	0.12–2.00 0.73 ± 0.52
Latency (s)	1.31–1.89 1.58 ± 0.16	1.75–2.72 2.13 ± 0.21
Area (mV/s)	0.22–4.18 1.38 ± 0.81	0.11–3.10 0.87 ± 0.65
	Sound stimulation	
Amplitude (mV)	0.12–3.40 0.99 ± 0.89	0.14–3.01 0.56 ± 0.51
Latency (s)	1.15–1.94 1.56 ± 0.23	1.27–2.64 2.09 ± 0.28
Area (mV/s)	0.19–4.72 1.14 ± 1.08	0.13–3.61 0.66 ± 0.68
	Electrical and sound stimulation	
Amplitude (mV)	0.20–2.24 1.15 ± 0.60	0.09–3.06 0.71 ± 0.58
Latency (s)	1.18–1.80 1.51 ± 0.14	1.65–2.61 2.09 ± 0.24
Area (mV/s)	0.22–5.32 1.44 ± 0.92	0.09–3.83 0.84 ± 0.71

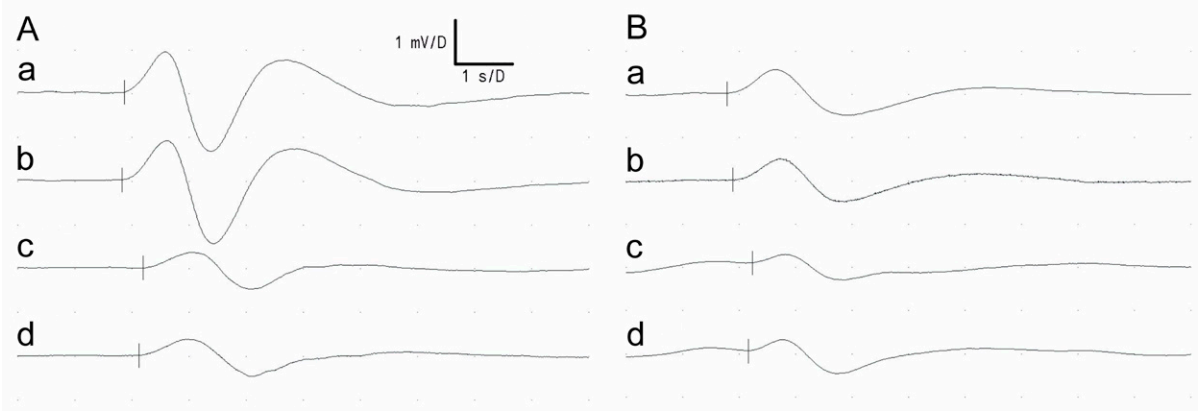

Figure 2. Examples of sympathetic skin responses recorded in two of healthy volunteers when evoked with (A) electrical stimulus and (B) sound stimulus. Recordings were acquired from right and left upper (a, b) and lower extremities (c, d)

Table 3. Differences in parameters of SSR evoked potentials with three types of stimuli. Asterisks (*) indicate the statistically significant differences at $p \leqslant 0.05$

Parameter	p	
	Upper extremities	Lower extremities
Amplitude (mV)	0.062	0.081
Latency (s)	0.105	0.495
Area (mV/s)	0.011*	0.023*

Table 4. Comparison of mean values of percentage changes in RRIV tests obtained in this and other authors studies

	This study	Other studies
Normal breathing	24.45	18.91; 18.62
Deep breathing	37.95	31.01; 31.42
Normal-deep breathing difference	13.5	12.11; 13.72

[1] Shahani et al. 1990, [2] Ozgocmen et al. 2006

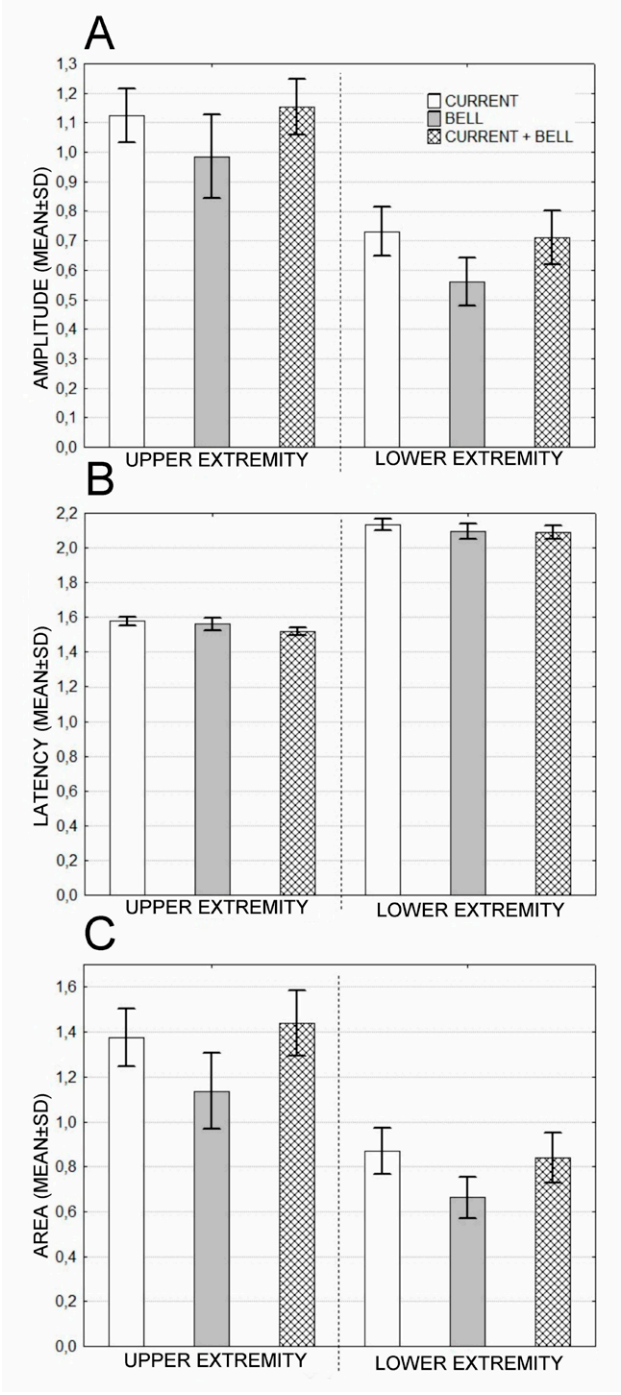

Figure 3. Histograms showing distribution of mean values and standard deviations with reference to parameters of amplitudes (A), latencies (B) and areas (C) of recorded SSR from upper and lower extremities when evoked with three types of stimuli. Note similar latencies but variability of amplitudes influences the area parameter in sound stimulations

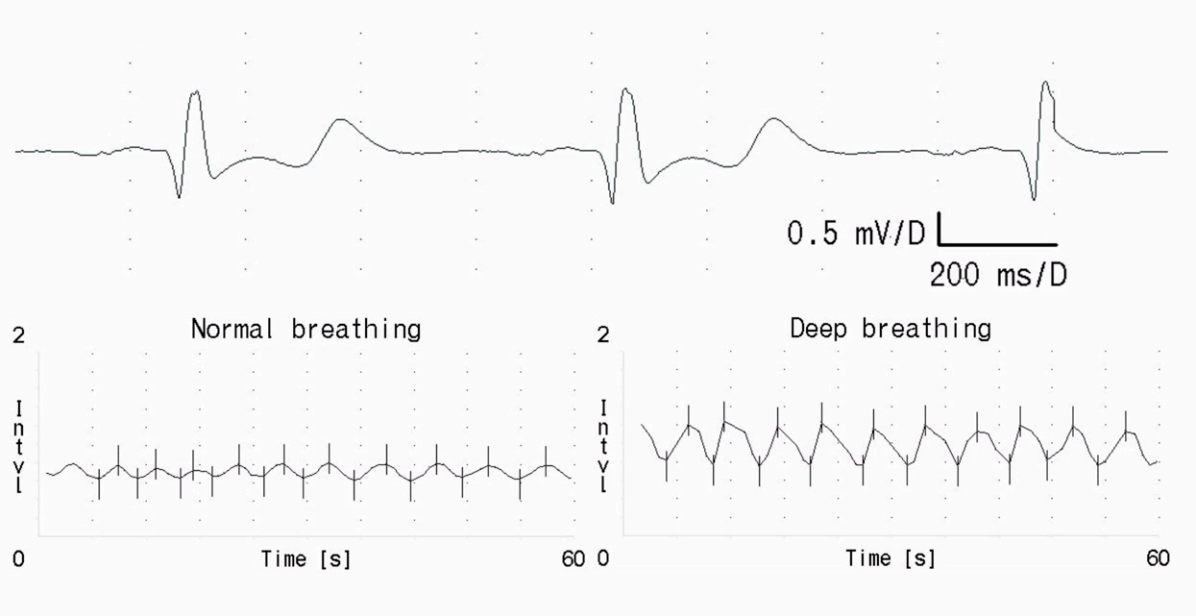

Figure 4. Examples of RRIV recordings performed in one of examined volunteers during normal and deep breathing

stimulation when recorded from both upper and lower extremities. The standard deviation for the SSR amplitude recorded during three types of stimulation was close to the range 0.5–0.6 mV. Thus, it can be assumed that bell ring stimulus evokes the response with lower values of amplitude which can be probably explained by weaker excitation of the sympathetic system than the one with the electrical stimulus.

Examples of proper RRIV recordings during normal and deep breathing are presented in **Figure 4** while their principles are presented in **Table 4**. During deep breathing the increase in heart beats was predominantly observed (in 70% of examined persons). The percentage difference of heart beats variability changed by 13.5% which was parallel with the RRIV amplitude increase during the deep breathing.

Discussion

One of the aims of this paper was the analysis of the influence of the different from standard sound and electrical stimuli on the parameters of evoked SSR. Similar studies have not been performed according to the data available from literature. Only the work of Elie et al. [9] describes a similar combination of the two types of stimulation to induce SSR. However, they have used different values of the stimulus than in our research. Bursts of bi-aural stimuli with intensity at 85–105 dB and electrical stimulation at 0.85, 1 and 1.15 times threshold of the median nerve excitability were used, evoking responses with similar values as in our research. Using of electrical stimulus with intensity not exceeding 10 mA was also intentional in our tests. The stimulus with such values eliminates the possible modulation of the evoked response to stimulation of endogenous origin, such as pain, movement or sudden breath. Taking into account the comparison of results, obtained SSR recordings with electrical, other defined as bell ring and the combination of both stimuli had similar parameters. Mean values of SSR latencies evoked with defined electrical stimulus in our study are comparable to the results obtained by other authors [5, 14–16], which is presented in **Table 5**. Shahani at al. [13] and Levy et al. [17] described results of SSR evoked with undefined stimulus taking the origin from deep breathing. They recorded parameters of latencies similar to the ones observed in our sample when defined bell ring stimulus was applied.

During SSR recordings, the moment of defined bell ring stimulus applied simultaneously to the recording onset is methodologically crucial for obtaining the responses with similar latencies. This condition was rigorously kept during SSR tests performed in this study. The differences of latencies values which ranged at ±0.2 s for recordings obtained from upper and lower extremities were not significant. On the other hand, the latencies of SSR responses recorded following excitation with sound stimulus and combined electrical-sound stimuli were very close to those evoked by electrical stimulus only. The centripetal conduction time is depended on the stimulus type, however, it constitutes only 5% of SSR latency. This phe-

Table 5. Comparison of mean values of latencies (in seconds) from SSR recordings evoked with electrical stimulus during this study to latencies of SSR in studies of other authors

Recorded extremities	This study	Studies of other authors
Upper	1.58 ± 0.16	1.391; 1.322; 1.433; 1.214; 1.55
Lower	2.13 ± 0.22	1.881; 1.722; 2.233; 1.864; 2.25

[1] Shahani et al. 1984
[2] Zakrzewska-Pniewska et al. 1998
[3] Cariga et al. 2002
[4] Ozgocmen et al. 2006
[5] Łabuz-Roszak et al. 2009

nomenon does not influence the final result of latency value. The comparison of our data on SSR latency with the results of other authors is not possible because of any particular data on this problem except for the work of Elie and Guiheneuc [9] who used the biaural tone burst for stimulation. They also did not observe changes in SRR latencies evoked with bimodal and electrical stimulation.

Similarly to the studies of Shahani et al. [5], SSR recordings from upper extremities were with higher amplitudes than those recorded from lower extremities during the application of electrical stimulation. Moreover, in our study a similar phenomenon was observed for a combined type of stimulation. During the application of bell ring the mean value of amplitude was lower both for upper and lower extremities than in other types of stimulations (**Figure 3**). The value of bell ring at 80 dB (40–50 dB above the hearing threshold) evokes SSR with optimal amplitude (> 180 μV recorded from palm, > 160 μV recorded from foot). The bell ring stimulus applied in our study beside other parameters and different technique, evoked SSR with similar latency values as reported by Kucera et al. [18] but the amplitude was lower. Greater variability of amplitude and area parameters can be also influenced by the habituation phenomenon [1, 5, 6, 18]. It can be concluded that latency parameter is more objective in SSR recordings for the evaluation of autonomic nervous system function than amplitude. There were not changes in values of this parameter in the studies of other authors [5, 19–22] who stimulated nerves with intensity in the range from 10 to 60 mA. Stimulation of nerves other than median (tibial, peroneal and supraorbital nerves) did not influence SSR parameters [14, 23, 24].

Gender differences were not observed during the analysis of SSR parameters in our studies. Basing on the descriptions of other authors, height and body mass did not influence SSR responses [6, 17]. However, changes in their parameters in 50% of the people after 60 were observed [18, 25]. These data cannot be verified by our studies because of our sample homogeneity. The lack of SSR recording can be a sign of dysautonomia as well as their unilateral recording or absolute value of latency more than ±2SD. Routinely applied both types of stimuli (sound or electrical) during SSR evaluation may reveal the level of pathology origin with respect to the afferent routes of impulses transmission.

Mean results obtained from RRIV recordings are comparable to those obtained by other authors (**Table 5**). R-R variability during the deep breathing and the heart beat acceleration were observed in 18 out of 20 of the examined volunteers. The diagnostic sensitivity of this test might have been proved by no false results during the examination of young, healthy volunteers. Repeatability, short duration (2 minutes) and non-invasive nature of this study are its advantages.

Attempts are made to use SSR and RRIV tests in confirmation of spinal muscular atrophy, progressive autonomic failure, fibromyalgia, uraemia, diabetes, polyneuropathies, carpal tunnel syndrome, disturbances in patients with Parkinson's diseases or epilepsy diagnoses [13, 15, 16, 19, 21, 22, 26]. No SSR responses during the application of both types of stimuli (sound and electrical) should be considered in confirmation of dysautonomia coexistence.

Similar studies comparing results from population of young and elder people would profit on the topic of using electrical and sound defined stimuli as triggers of SSR. Several neurological diseases affect rather older people and the parameters of the vegetative responses may change with age.

Conclusion

In conclusion, the various modalities of stimuli used in our studies evoke SSR with comparable values. However, in our opinion, the most objective parameter is a stable value of the SSR latency, regardless of the type of applied stimuli. Using of both electrical and sound stimuli is very important because it allows differentiation of abnormal SSR responses associated with the possible peripheral neuropathy. Compilation of SSR studies using different modalities of stimuli, as well as clinical and RRIV examination may influence the precision of dysautonomia diagnosis.

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Conflict of interest statement
The authors declare no conflict of interest.

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Correspondence address:
Prof. Juliusz Huber MSc PhD
Department of Pathophysiology of Locomotor Organs
Poznan University of Medical Sciences
135/147 28 Czerwca 1956 St, 61-545, Poznań, Poland
phone/fax: +48618310230
email: zpnr@wp.pl

REVIEW PAPER

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Structural and lectin-detectable changes of liver induced by a long-term administration of antihistamine agent Loratadine

Olga Dudok, Alexander Lutsyk

Department of Histology, Cytology and Embryology, Danylo Halytsky Lviv National Medical University, Ukraine

ABSTRACT

Adverse effects of pharmacological agents are currently under considerable attention of theoretical and clinical medicine. The aim of present investigation was to study in experiment the effect of a long-term administration of antihistamine drug Loratadine on the micromorphology and carbohydrate determinants of the liver. Experimental rats once a day during 30 days intragastrically received Loratadine in the form of an aqueous suspension in the dose of 0.15 mg/kg body weight. On days 10, 30, 40, 50, 60 of experiment euthanasia was carried out, liver samples excised, fixed in 4% formaline and embedded in paraffin. Examination of haematoxylin and eosin stained sections revealed extension of sinusoid capillaries and central veins, granular dystrophy of hepatocytes supplemented with perivascular lymphoid infiltration, these phenomena receiving highest development on days 30th and 40th of experiment. At the same times PAS reaction demonstrated increased content of glycogen deposits in hepatocytes, apparently encompassing alterations in glucose metabolism. On the later stages of experiment (days 20th and 30th after the last Loratadine injection) signs of hepatocyte alteration decreased significantly, and content of glycogen returned to normal values. Loratadine administration induced the accumulation in the intra- and 2 perisinusoidal space of hepatic lobules of activated Kupffer cells, which were strongly PAS- and WGA-positive, as well as the enhanced exposure of DGlcNAc and DGalNAc determinants by glycoconjugates in within the altered hepatocytes. Our results indicate certain destructive effect of Loratadine on hepatic micromorphology and function; the cessation of this drug administration was accompanied by simultaneous strengthening of regenerative processes.

Keywords: rat liver histology, lectin histochemistry, H1-blocker Loratadine.

Introduction

Nowadays more than 63 thousand chemical compounds are used by modern civilization, 55 thousand of which, including different kind pharmacological agents, can be dangerous to human health. Every year more than 1 million people suffer from the side effects of pharmacotherapy, resulting economic losses estimated as 136 billion dollars [1]. Since liver takes central position in the metabolization of xenobiotics and drugs, this organ most oftenly is subjected to injuries [2, 3]. The leading mechanisms of these lesions development is direct action of a pharmacological agent or of its reactive metabolites on the liver cells that leads to their death by necrosis or induction of apoptosis [4, 5].

Recently it was reported of possible toxic effects on the human organism of antihistamine agents, which are widely applied in the treatment of patients with various allergic conditions [6]. Patients are oftenly using these drugs without consulting a doctor. It was documented the use of antihistamines as components of drug mixtures and acute poisoning by them of drug-abusers [7].

Moreover, investigations are conducted on possible influence of such drugs on the health of employees in pharmaceutical industry, directly involved in their production [8].

In the available literature we find no data on possible hepatotoxic effect of antihistamines leading to the structural reorganization of liver, including its carbohydrate determinants. Meanwhile, their lectin histochemistry investigation may be of practical interest due to important role played by cell surface, cytoplasmic and 3 extracellular glycoconjugates in normal histophysiology; carbohydrate determinants are among the first to be damaged during pathological lesions development [9–12]. Therefore the aim of present investigation was to study long-term influences of Loratadine – H1-histamine receptor blocker – on hepatic tissues micromorphology and lectin receptor sites.

Material and methods

Experiments were carried out on 60 mature male Vistar rats 160–200 g of weight, kept under standard conditions of vivarium. All manipulations were performed in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986) and the law of Ukraine "About protection of animals from cruel treatment" (No 1759-VI of 15.12.2009).

Animals were subdivided into two groups: the first group (20 rats) served as a control; second group animals (n = 40) once a day during 30 days intragastrically received Loratadine in the form of an aqueous suspension in the dose of 0.15 mg/kg of body weight. Loratadine is a second generation H1-histamine receptors blocker, chemically designated as "Ethyl ether -4–8-chloro-5,6-dihydro-11H-benzo-[5,6]cyclohepta-[1,2-b]pyridin-11-len)-1-piridincarbonic acid", produced by "FARMACHEM SA Chem Limited" (India). The administered dose corresponded to the average daily therapeutic dose for humans. On 10th and 30th days of drug administration, and on days 40s, 50s and 60s from the beginning of experiment, animals were subjected to euthanasia with subsequent dislocation of cervical vertebrae; thereafter liver samples were collected for morphological studies.

For routine histological examination obtained material was fixed in 4% neutral formalin and embedded in paraffin; sections 5–7 μm thick were stained with haematoxylin and eosin. For getting semi-thin sections tissue samples were fixed in 2% OsO4, dehydrated and embedded into epon-araldite; 1 μm sections were cut using ultratome YMPT-3M, subjected to PAS reaction and counterstained with methylene 4 blue [13].

Carbohydrate determinants of hepatic tissues were detected by a set of 7 lectins (**Table 1**). All used lectins were purified and coupled to horseradish peroxidase in the laboratory by Dr. Pharm. Sci. V.O.Antonyuk. Lectin receptor sites were visualized in PBS, containing 0.05% diaminobenzidine (Sigma, St.Louis, USA) and 0.015% H2O2 as described elsewhere [14]. Slides were studied and pictures taken using Leica DM 2500 microscope equipped with Leica DFC 450C digital camera.

Results

Examination of control specimens stained with haematoxylin and eosin revealed typical rat liver micromorphology (**Figure 1**), in comparison to which liver of Loratadine treated animals exposed certain signs characteristic for drug metabolization. Namely, on the experimental day 30th sinusoid capillaries expansion was accompanied with periportal lymphoid infiltration (**Figure 1B**). On the day 40th increased density of hepatocytes cytoplasm and nuclei was associated with decomplication of hepatic plates and sludge phenomena in within the expanded sinusoids (**Figure 1C**). Hepatocytes of the same group animals exposed signs of granular degeneration, hydropic dystrophy and nuclear pyknosis (**Figure 1D**).

Table 1. Lectins used and their carbohydrate specificity, according to data of V.O. Antonyuk [15]

No	Lectin name and abbreviation	Carbohydrate specificity	Complementary oligo-/ polysaccharide
1	Peanut agglutinin, PNA	βDGal(1-3)DGalNAc	Thomsen-Friedenreich antigen
2	Laburnum anagyroides bark agglutinin, LABA	LFuc	Gal(β1-4)Fuc(β1-3)Glc
3	Sambucus nigra agglutinin, SNA	NeuNAc(α2-6)DGal	NeuNAc(α2-6)Gal(β1-4)GlcNAc(β1-2)
4	Wheat germ agglutinin, WGA	DGlcNAc > NeuNAc	NeuNAc(α2-6)Gal(β1-4)GlcNAc, Man(β1-4) GlcNAc(β1-4)GlcNAc
5	Galanthus nivalis agglutinin, GNA	αDMan	Man(α1-3)Man(β1-4)GlcNAc
6	Clitocybe nebularis fungus agglutinin, CNFA	DGalNAc(β1-4)GlcNAc	Not estimated
7	Helix pomatia agglutinin, HPA	αDGalNAc	GalNAc(α1-3)GalNAc

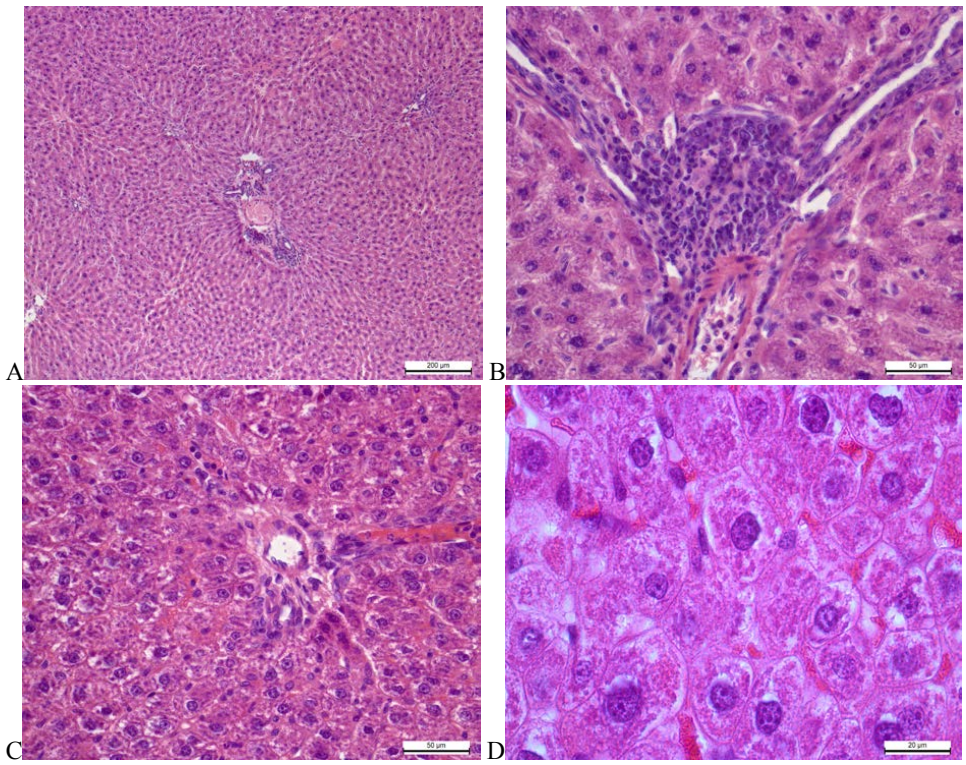

Figure 1. Changes in rat liver micromorphology induced by a long-term Loratadine administration. A. Liver of intact rat; B. Experimental day 30th: perivascular lymphoid infiltration; C. Experimental day 40th : extension of sinusoid capillaries, hepatic plates decomplication; D. Hepatocytes on the experimental day 40th: signs of granular degeneration, hydropic dystrophy and nuclear pyknosis. Haematoxylin and eosin, original magnification x100(A), x400(B, C), x1000(D)

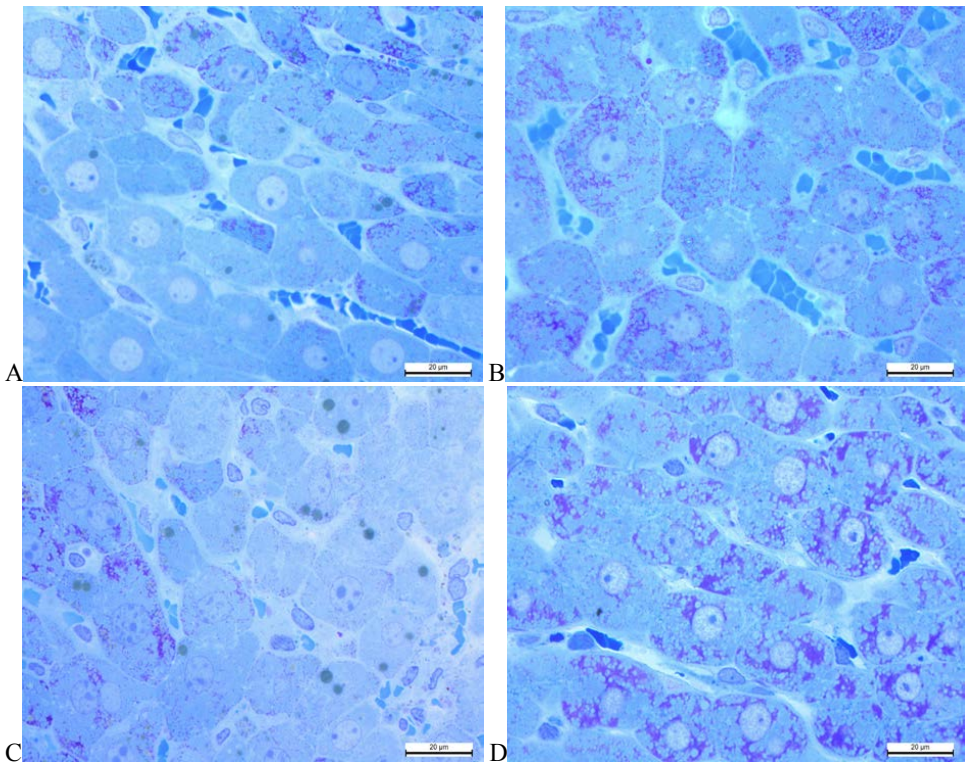

Figure 2. Semi-thin sections, PAS-reaction with methylene blue counterstaining. A. Liver of intact rat: dark blue coloration of small size Kupffer cells with intra- and perisinusoidal localization. B. 30th day of Loratadine treatment: accumulation of fine glycogen granules in the cytoplasm of hepatocytes. C. Experimental day 40th: densely packed glycogen inclusion overload groups of dystrophic hepatocytes; D. Experimental day 60s: amount of glycogen inclusions close to normal, intra- and perisinusoidal accumulation of Kupffer cells. Original magnification x1000 (A-D)

Semi-thin sections of liver samples of control rats, as well as of experimental animals at early stages of Loratadine treatment demonstrated typical polygonal shape hepatocytes, endotheliocyte lined sinusoids, with few small densely stained cells with intra- and perisinusoidal localization – apparently Kupffer and Ito cells (**Figure 2A**). On the experimental day 30th the accumulation of fine glycogen granules was detected in the hepatocytes (**Figure 2B**); on day 40th these densely packed glycogen inclusions overloaded groups of hepatocytes, apparently encompassing their granular dystrophy (**Figure 2C**). Up to experimental day 60s the amount of glycogen inclusions decreased significantly, while number of Kupffer and Ito cells in within the hepatic lobules increased (**Figure 2D**).

Used lectins showed rather differential binding to carbohydrate determinants of 5 hepatic tissues in control and experimental animals. Most informative results were obtained with WGA, CNFA and HPA. Namely, in control rats WGA strongly labeled endothelial cell lining of liver sinusoids, cytoplasm and nuclei of hepatocytes being completely non reactive (**Figure 3A**). After 10 days of Loratadine administration this lectin reactivity was detected in the cytoplasm of small cells with periportal localization (**Figure 3B**). Number of these cells increased in intra- and perisinusoidal spaces on days 30th of experiment; similarly increased the exposure of WGA receptor sites within the cytoplasm of hepatocytes (**Figure 3C, D**).

CNFA in the liver of control rats selectively labeled bile capillaries and plasma membranes of hepatocytes, other hepatic elements being areactive (**Figure 4A**). In the liver of early stage experimental animals this lectin binding was restricted to cytoplasmic and nuclear glycoconjugates of hepatocytes and central veins endothelium (**Figure 4B**). On experimental days 40s CNFA binding was additionally detected within the cells of intra- and perisinusoidal localization, apparently activated Kupffer and Ito cells (**Figure 4C**). On days 60s nuclei and plasma membrane of hepatocytes demonstrated enhanced lectin reactivity (**Figure 4D**).

HPA binding in the liver of control rats was restricted mostly to the luminal surface of sinusoid capillaries and plasma membranes of hepatocytes (**Figure 5A**). Among the most interesting findings was this lectin strong reactivity with cytoplasmic glycoconjugates of plasma cells (**Figure 5B**), which were previously identified in within the hepatic lobules by means of electron microscopy [16]. Loratadine administration induced significant accumulation of HPA receptor sites in the

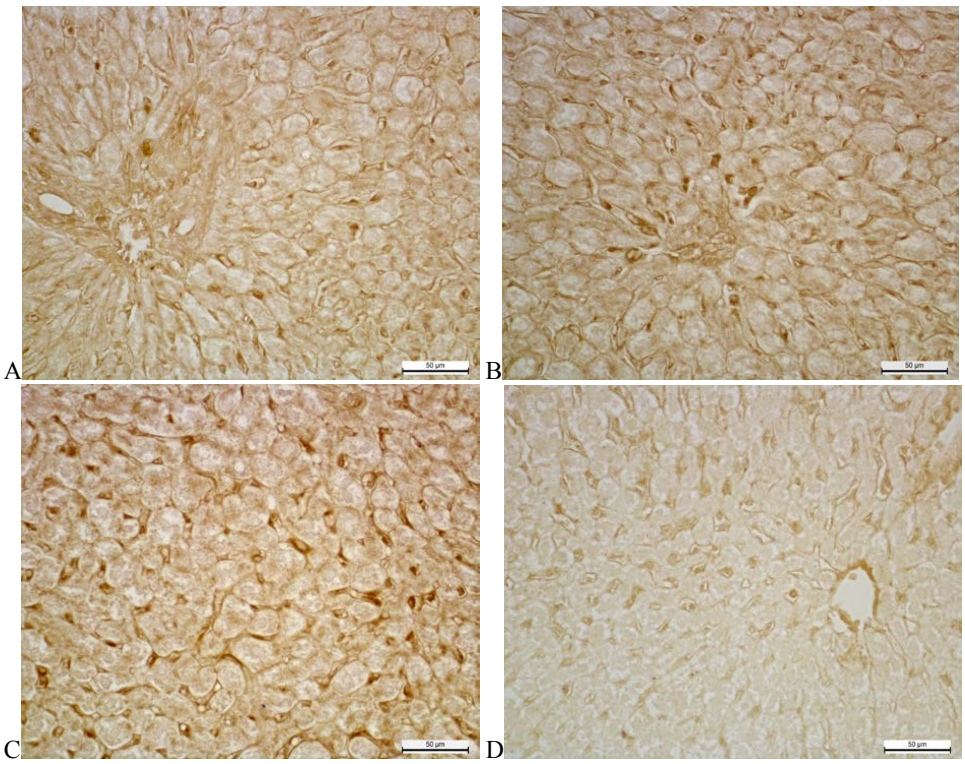

Figure 3. Redistribution of WGA receptor sites after Loratadine administration: liver of intact rat (A), on experimental days 10th (B), 40s (C), and 50s (D). Lectin label restricted to few Kupffer cells in periportal area (A), surrounding central vein (B); increased number of strong WGA-positive cells with intra- and perisinusoidal localization (C, D); enhanced reactivity of hepatocytes (C) and of central vein endothelium (D). Original magnification x400 (A–D)

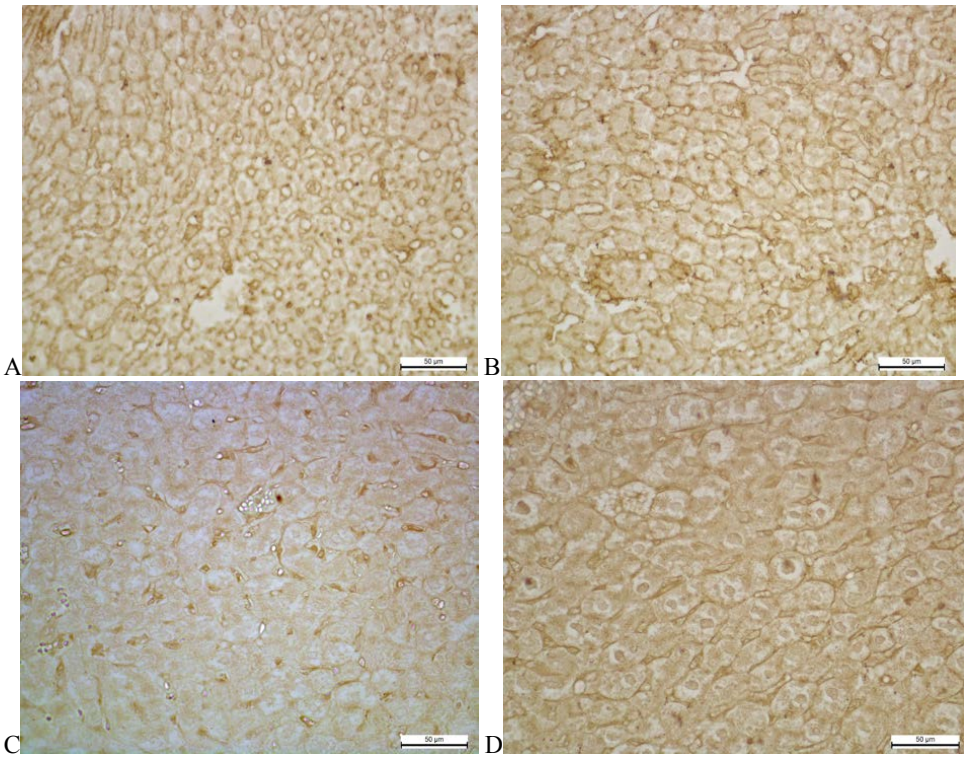

Figure 4. CNFA binding to liver of intact rat in comparison to liver after different terms of Loratadine administration. A. Liver of intact rat: lectin labeling of bile canaliculi and plasma membranes of hepatocytes. B. Experimental day 10th: enhanced reactivity of hepatocytes nuclei. C. 40s day of experiment: reduced reactivity of hepatocytes cytoplasm, bile canaliculi strong positive. D. 60s day of experiment: increased reactivity of hepatocytes cytoplasmic and nuclear glycoconjugates. Original magnification x400 (A-D)

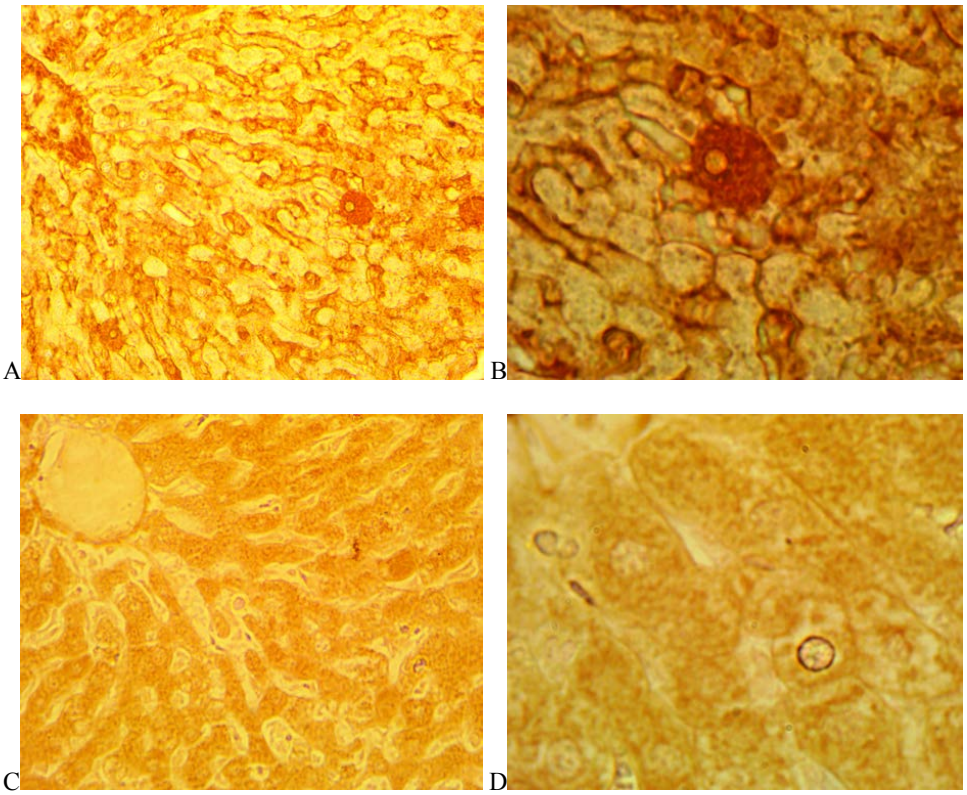

Figure 5. HPA label in hepatic tissue of control rat (A, B) and its redistribution on day 30th of Loratadine treatment (C, D). A. Intense reactivity of central vein endothelium, sinusoid capillaries, and cytoplasmic glycoconjugates of few plasma cells. B. Plasma cell in between plates of hepatocytes. C, D. Enhanced reactivity of cytoplsmic glycoconjugates in within the hepatocytes. Original magnification x400 (A, C), x1000 (B, D)

cytoplasm of hepatocytes, this redistribution being especially prominent on day 30th of experiment (**Figure 5C, D**). With the rest of lectins used (PNA, GNA, SNA and LABA) no remarkable differences were found in the labeling of liver structures in between control and Loratadine-treated rats.

Discussion

Our results demonstrate that long-term daily administration of Loratadine induce 6 changes in liver micromorphology, highest manifestations of which account for 30th–40s days of experiment. These changes gradually decrease up to experimental day 60s (till the 30th day after last Loratadine administration). Detected signs of granular, and, in parts, hydropic dystrophy, apparently encompass transient rearrangement of biosynthetic function of hepatocytes into metabolic function – a phenomenon reported by Yvashkin et al. [17], directed towards Loratadine metabolisation. Lymphoid infiltration of perivascular spaces most likely is indicative of antigenic stimulation in response to spontaneous conjugation of Loratadine metabolisation products with endogenous proteins resulting with the formation of haptens [18].

Rather unexpected was our finding on the ability of Loratadine to induce the accumulation of glycogen in hepatocytes. Dual interpretation of this phenomenon is possible: (1) some reactive metabolites of Loratadine can serve as triggers of glucose metabolic processes impairments, directing them towards enhancement of gluconeogenesis (this mechanism was reported by Yakovleva et al. [19] for metabolisation of xenobiotics); (2) a long-term blockade of H1-histamine receptors in hepatocytes (presence of which was documented by Camelo-Nunes [20]) affects the activity of intracellular enzymes (adenilatcyclase, cAMP), involved in the regulation of gluconeogenesis. The important role of these enzymes in the regulation of glucose metabolism under the influence of antihistamines was reported by Berezhnaya et al. [21].

On the experimental days 50s and 60s it was detected significant decrease of dystrophic changes in hepatocytes accompanied with the accumulation of Kupffer and Ito cells in within hepatic lobules. These observations apparently encompass reduction of the alterative processes with simultaneous strengthening of the regenerative mechanisms. In particular, according to Berezhnaya et al. [21] and Takeishi et al. [22], Kupffer cells stimulate regeneration of hepatocytes by enhanced expression of hepatocyte growth factor via TNF2-independent mechanism, while cells of Ito are stimulating neovasculogenesis in clusters of regenerated hepatocytes [23].

Our findings on lectin reactivity of hepatic tissues are consistent and extend our previous observations on the redistribution of liver carbohydrates in streptozotocin-induced diabetes mellitus [24]. We assume that detected accumulation of WGA, CNFA and HPA receptor sites (DGlcNAc and DGalNAc determinants) within the hepatocytes apparently encompass transient Loratadine induced alterations of these cells synthetic machinery. Among possible mechanisms causing this phenomenon can be inhibitory effect of used antihistamine drug on carbohydrates final glycosylation steps in within Golgi complex, or inhibition of glycoconjugates exocytosis via plasma membrane of affected hepatocytes.

Lectins proved their usefulness as selective histochemical markers of normal rat liver structures: WGA – of vascular endothelium; CNFA – of bile canaliculi and plasma membranes of hepatocytes; HPA – of hepatic plasma cells. After Loratadine administration WGA intensely labeled cells with intra- and perisinusoidal localization – apparently activated Kupffer cells. Our recent results somewhat disagree with earlier observations of Yashchenko et al. [24] on strong PNA reactivity of rat Kupffer cells; however, these discrepancies should be investigated in future research.

Conclusions

Our studies demonstrate that prolonged use of H1-histamine receptors blocker Loratadine induce transient changes, which reflect shifting of hepatocytes biosynthetic activity to drug metabolisation. Although causing granular and, partly, hydropic dystrophy, as well as the accumulation of glycogen deposits in hepatocytes, detected changes do not lead ultimately to these cells necrosis or apoptosis. However, the drug can be potentially dangerous with concomitant liver disease (HCV and HBV, HIV infections, etc.). Loratadine administration induced redistribution of WGA, CNFA and HPA receptor sites within the hepatocytes (exposure of DGlcNAc and DGalNAc determinants), apparently encompassing alterations of these cells carbohydrate synthetic machinery. Development of antigenic stimulation was reflected by the lymphoid infiltration of periportal areas, accumulation of activated 8 Kupffer and Ito cells in intra- and perisinusoidal spaces. Lectins can be recommended for selective histochemical labeling of rat liver structures: WGA –

of vascular endothelium, activated Kupffer cells; CNFA – of bile canaliculi; HPA – of hepatic plasma cells.

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Conflict of interest statement
The authors declare no conflict of interest.

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Correspondence address:
Olga Dudok
Department of Histology, Cytology and Embryology
Danylo Halytsky Lviv National Medical University 69
Pekarska St, 79010 Lviv, Ukraine
phone: + 38 0985505304
email: dudok.olga@gmail.com

ORIGINAL PAPER

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Assessment of static loads on the locomotion system accompanying work on dairy stock farms

Bartosz Bilski

Department of Preventive Medicine, Poznan University of Medical Sciences, Poland

ABSTRACT

Aim. The aim of this study was to perform statistical analyses of the load on the locomotion system during a variety of basic (elementary) work tasks associated with dairy cattle breeding, its causes and suggested key preventive measures illustrated, with an example of a large dairy stock farm.

Materials and methods. A comparative analysis was performed to compare the activities of the locomotion system during the use of traditional and modern milking methods. The analysis included elementary work tasks performed by 12 healthy, full-time stock workers (only males) employed at a large dairy stock farm in the Province of Wielkopolska, operating as a limited liability company. The working area consisted of two dairy cowsheds, in which different milking methods were used. OWAS (Ovako Working Posture Analysing System) method and the supporting WinOWAS computer system were employed to analyse all occupational activities generating static loads.

Conclusions. The elementary work tasks in dairy cow breeding may involve significant loads on the musculoskeletal system. Unergonomic performance of these tasks results from bad habits and the level of mechanisation specific to a dairy cowshed. The proposed corrective and preventive measures presented in the analysis of specific works consists mainly in substituting the tools used so far with more ergonomic equipment, which is safer for the human locomotion system. The implementation of the proposed solutions requires specific investments; however, the risk of locomotor system disorders can be significantly reduced. Specific works, especially in traditional cowsheds, such as cow preparation for milking and the milking process itself, require prompt corrective measures, however, the lack of space may seriously limit the possibility to implement such measures, and stock workers are forced to assume awkward body positions. Education of stock farm staff should become one of the key preventive measures. Educational campaigns should be introduced within the framework of obligatory occupational safety training, in particular. However, the access to occupational safety training among individual farmers in Poland is currently very limited and may pose a challenge.

Keywords: static loads, OWAS, dairy stock farms, musculoskeletal system.

Introduction

In the majority of occupational conditions in agriculture, the locomotion system is exposed to specific loads [7, 9, 13–15, 17–20, 31], such as physical effort to compensate static load, which may negatively affect general health, and the locomotion system in particular [2, 13, 26, 30, 35, 40–44]. The operation of milking equipment is another risk factor for injuring the wrists and hands [38]. Typical working activities in agriculture, especially activities associated with the breeding of dairy cattle, constitute major risk factors for low back pain (bending, twisting, manual material handling and exposure to whole-body vibrations, etc.), neck and shoulders symptoms (especially monotonous and repetitive work) and osteoarthritis of the hip and the knee (proposed as a contributing factor) [1, 6, 21, 27, 29, 32, 35, 41, 46]. The outcomes of previous studies show that pre-milking, attaching and drying (cleaning the udder with a towel) were the most physically demanding milking tasks for the wrists and hands [27,

32, 38]. Another major problem mentioned in the available literature is the effect of technical equipment in breeding and milking premises on the level of physical load the locomotion system is exposed to in individuals working with dairy cattle [2, 32, 37, 38].

Aim

The aim of this study was to perform statistical analyses of the load on the locomotion system during a variety of basic (elementary) work tasks associated with dairy cattle breeding, its causes and suggested key preventive measures illustrated with an example of a large dairy stock farm. A comparative analysis was performed to compare the activities of the locomotion system during the use of traditional and modern milking methods.

Materials and methods

The analysis included elementary work tasks performed by 12 healthy, full-time stock workers (only males) employed at a large dairy stock farm in the Province of Wielkopolska, operating as a limited liability company. Their mean age was 41 years (range 24–49) and their median height and weight were 173 cm (range 158–180) and 79 kg (range 59–87), respectively. All workers were right handed. Each worker was recorded during the entire duration of elementary work tasks. The working area consisted of two dairy cowsheds, in which different milking methods were used. OWAS (Ovako Working Posture Analysing System) method and the supporting WinOWAS computer system were employed to analyse all occupational activities generating static loads [45]. This software is available for free online [45]. The OWAS method has been used quite commonly and offers clearly specified operating procedures, hence the analysis outcomes are easier to discuss and extrapolate as compared to the outcomes of the author's own research methods [4, 5, 10, 12, 16, 22–25, 36]. In order for the presented outcomes to be as universal as possible, the total daily and weekly timing of the performed activities was excluded from analysis, and the analysis was focused predominantly on the assessment of static loads during the so-called elementary work tasks. Note that the total risk assessment of locomotor system complaints may differ depending on the total time devoted to the performance of specific elementary work tasks (note that the majority of Polish farms are privately owned, and the number of working hours differs significantly from one farm to another).

The study materials (video recordings) were registered by means of a video camera on site, and were later used in further analyses. The stock workers were video recorded performing specific elementary tasks composed of specific activity cycles. Whenever a single activity within the framework of a specific task was difficult to separate and to record (whenever specific activities involved frequent changes in the position of particular parts of the body), several activity cycles were analysed together. This was done to generate averaged, real study results.

The study algorithm of the OWAS method consists of:

- video recording of a working cycle (work task),
- analysis of the video recordings, consisting in the assessment of baseline body position and external load (which was classified and assigned a specific code according to the OWAS notation, **Table 1**) and subsequent assessments of each change in a specific body part position (upper and lower extremities, trunk) and the level of external load. The evaluation outcomes are reported in a standard report form

Table 1. Categories of static load size according to OWAS [45]

Category	Description
1	– natural position/s during work – optimum or acceptable load – no changes are required at the workstation
2	– position/s during work may negatively affect the musculoskeletal system – loads close to acceptable – there is no need for immediate changes at workstation, however, they need to be considered in the near future
3	– position/s during work negatively affect the musculoskeletal system – high loads – changes at workstation need to be introduced as soon as possible
4	– position/s during work have a powerful negative effect on the musculoskeletal system – very high loads – changes at workstation need to be introduced immediately

– data from report forms (separate for each work task) entered in WinOWAS. On the basis of data entered in WinOWAS, the software generates a collective list of codes for the observed body positions, which are assigned to specific postural load categories (**Table 1**). The percentage share of the analysed work tasks assigned to the respective categories reflects the level of static load during specific elementary work task, and may be used in further analyses of the total risk of musculoskeletal complaints when performing a series of elementary work tasks throughout a working day. The analysis outcomes are also presented graphically as diagrams of loads on specific body parts and the share of each category in the total workload in the course of elementary work tasks (these data were omitted as too elaborate). These data were sufficient to determine, which elementary work tasks involved high peak static loads and to specify body parts most exposed. On the basis of the study outcomes, specific body postures were determined as preventive measures, to be introduced in conjunction with applied corrective ergonomics.

RESULTS

During a typical working day, the following 9 elementary work tasks were observed and video recorded:

– manual removal of manure from cow stalls,
– removal of manure from the dairy cowshed with a farm tractor equipped with fore loader,
– cleaning of cow stalls with a pressure washer,
– manual spread of litter,
– manual sweeping of feed in the dairy cowshed,
– cow preparation before milking in a traditional dairy cowshed – udder hygiene,
– preparation of cows before milking in a traditional dairy cowshed – milk sampling,
– milking in a traditional dairy cowshed,
– milking in a new dairy cowshed,

The work tasks were selected based on the criteria of significance and autonomy. As a result, the analysis included elementary work tasks routinely performed by stock workers that consumed the majority of working time, and on the other hand – tasks that were significantly distinct. For example, in terms of static load, the removal of manure with a farm tractor is similar to feed supply with a tractor drawn feed carrier. Hence, detailed analysis included only activities performed in the course of manure removal, however, the analysis results reveal musculoskeletal load typical for both tasks. These similarities were indicated in the description of specific elementary work tasks.

Elementary work tasks

*Manual removal of manure from cow stalls (**Figure 1, 2**)*

This work task consisted in the removal and scraping of manure from cow stalls to a 10-cm deep and 80 cm wide manure passage adjacent to cow stalls. Manure was removed with a fork. Stock workers were video recorded when performing this task at 5 adjacent stalls. During the entire working day, a single stock worker removed manure from app. 40 similar cow stalls. In the course of manure removal by 3 stock workers, 22 changes in body positions or loads were observed (22 changes against 23 body positions).

Figure 1. One of body positions during manual manure removal from cow stalls

Figure 2. Another body position during manual manure removal from cow stalls

Related tasks included the collection and relocation of manure remaining after the passage of a tractor that removes the manure onto a heap or that loads the manure from the heap to a trailer or manure spreader. The analysis of video recordings revealed that the stock workers spent as much as 17% of their working time in body positions assigned to Category 4, which involves significant static load on the locomotor system, especially working with the trunk bent and/or twisted and/or with the weight of the body resting on one bent leg (even if the additional external load is limited). These body positions require prompt corrective measures. Also, various body positions assigned to Category 3 put a significant strain on the locomotor system and may cause lasting disorders if performed on a regular basis. In terms of specific body parts, manual manure removal involves significant loads on the trunk (as much as 35% of the working time is spent in bent and twisted body position, which is a major risk factor of lumbar spine disorders). Lower extremities are also severely strained, especially when the task is performed on one bent leg.

Removal of manure from dairy cowshed using a tractor with loader

This elementary task consisted in the removal of manure when driving along the manure passage with a farm tractor URSUS C-360 equipped with a fore loader TUR-2. This task can be divided into the following activities: removal of manure from the cow shed, manure storage outside the cow house on a manure slab, and driving the vehicle backwards back into the cow shed. Specific isolated activities were unrelated to specific distribution of isolated loads; therefore the analysis included all recordings. The duration of a single complete analysed activity cycle was 1 minute 8 sec on average, and involved 17 changes in the position of body parts.

Figure 3. One of body positions during stall cleaning

The analysis of video recordings revealed no body positions assigned to Category 3 and 4, which means that the static load in the course of this elementary work task was relatively low. The analysed activities were done in a sitting position, and the value of additional forces did not exceed 100 N, so the stock worker's body position was assigned to Category 2 due to awkward positions of the trunk (bent, or with the lumbar or (additionally) cervical spine simultaneously bent and twisted, especially when driving backwards). In addition, stock workers are exposed to whole-body vibrations, which is a risk factor for lumbo-sacral spine disorders. The related tasks may include feed preparation using a feed carrier and driving a feed carrier along the cow house.

Cleaning of cow stalls

This elementary work task consisted in the cleaning of cow stalls using Kärcher high-pressure washer. Stock workers were wearing wellingtons and waterproof overalls, and were controlling a washing pipe held in their hands and directed a stream of liquid to manure and litter remaining in the stalls. The aim of this task was to clean the cow stalls and to provide conditions for subsequent disinfection of the premises using chemical agents. The cleaning process of 2 stalls was video recorded, which took 1 minute and 34 seconds on average when performed by a single stock worker. During this time, 20 changes in the position of body parts were observed. In the course of this elementary task, objects up to 10 kg were carried by the stock workers. The analysis of video recordings revealed no body positions assigned to Category 3 and 4, which means that this elementary work task (cleaning the cow stalls with a pressure washer) involved no significant static load. However, over 3/4 of all body positions were assigned to Category 2 in terms of the static load as the stock worker's trunk was frequently bent forward, or simultaneously bent and twisted. There were two body positions when the stock workers were required to maintain one arm above the acromion (**Figure 3**). Related work tasks included floor cleaning in the milking parlour or work related to room disinfection.

Figure 4. One of body positions during litter spreading

Figure 5. Another body position during litter spreading

Figure 6. Another body position during litter spreading

*Spreading of litter (**Figures 4, 5, 6**)*

This elementary work task consisted in manual placing of litter in stalls using a fork. This task comprised the following activities: collecting straw from a bale, removal of straw to a cow stall typically located at a 10 m distance, litter spread in the cow stall. 5 cow stalls were video recorded, and each stock worker was typically in charge of 40 cow stalls per each working day. In the analysis of video recordings, 22 changes in the body position and load during this elementary work task were identified (the load meaning also the value of force used, for example, when the fork was inserted into a layer of baled straw).

As evidenced in the number of body positions assigned to the relevant categories, this elementary work task involved a considerable static load. Approximately 13% of the time devoted to this task accounted for body positions assigned to Category 4. As much as nearly ¼ of this time was spent in positions assigned to Category 3 (which may be also harmful). The collection of straw from a bale put the most static strain on the stock worker. Straw was collected on bent knees, and simultaneously bent and twisted trunk. Note that straw can be quite heavy when wet or tightly baled. As already mentioned, the peak static load is put on the trunk during this particular task (workers usually lean forward, or bend and twist the trunk, which involves high risk of injury).

*Manual sweeping of feed in the dairy cowshed (**Figure 7**)*

This elementary work task consisted in the handling and sweeping of feed in a feeding passage in the direction of cow stalls, using a 460 x 360 mm shovel. The feed was prepared in a feed wagon and consisted of a homogenous mixture. This elementary work task was done at approximately 80 cow stalls per one stock-worker per day. Stock workers performing this task were video recorded at 5 different cow stalls. As many as 33 changes in the positions of body parts or the external load values were identified. This task was very dynamic with frequent changes of body positions and sinusoidal distribution of loads (empty shovel-full shovel).

Approximately 1/2 of all body positions and loads during this work task were assigned to Category 3 and 4. These body postures require prompt corrective measures. Only 9% of body postures were assigned to Category 1. The highest strain was put on lower extremities during this particular elementary work task, as 44% of the time devoted to this task was completed on bent legs (the time interval when stock workers were holding a full shovel). The bent legs 'supported'

Figure 7. One of body positions during manual feed sweeping

the upper extremities. Workers found it easier to carry the load on and in front of the shovel while holding the legs bent. For over 90% of time, stock workers held their trunks bent forward, and simultaneously bent and twisted the trunk for as long as 1/3 of the time devoted to this task. Related tasks included manual loading activities, such as the removal of the remaining silage from the storage bins.

Cow preparation before milking in a traditional dairy cowshed – udder hygiene

This elementary work task consisted in udder cleaning with a cloth soaked in water with an addition of antiseptic agent before milking. This task was performed in a traditional cow shed with no milking parlour pit. Stock workers, who performed this task were squatting or kneeling in order to reach the udder. Apart from the cloth, stock workers needed to have 2 buckets, 10 l vol. each, filled with water solution with an addition of antiseptic preparation. There were two milking procedures performed each day. There were approximately 240 cows in the analysed cowshed, which means that the milking process was repeated 480 times every day. 4 stock workers were responsible for pre-milking udder hygiene, which means that each stock worker needed to clean 120 cows every day. The cleaning process took 2 minutes and 10 seconds on average to complete. 24 changes in the body parts were observed during this elementary work task. This task was mainly done at less than 10 kg load. Only when stockworkers carried buckets with water, the external load equalled app. 18 kg.

The number of body positions and the accompanying loads assigned to specific categories was similar, which means that the share of harmful activities assigned to Category 3 and 4 was significant. The activities assigned to Category 4 included body postures with the pectoral and lumbo-sacral spine simultaneously bent forward and twisted. Stockworkers were keeping their legs bent or were kneeling on one or both knees. In the latter case, the stockworker's body position was qualified to Category 3, as the static load was slightly lower, but still significant. In the position assigned to Category 3, the trunk was bent forward and one of the arms was raised above the shoulder joint. Related task consisted in applying an ointment on the udder in case of inflammation.

*Collecting milk samples in a traditional dairy cowshed (**Figure 8**)*

This elementary work task consisted in collecting milk samples from 4 teats onto a stand with 4 separate fields. The samples were collected from each cow to verify if the milk was fit for consumption and to prevent mixing high-quality milk with milk found unfit for consumption. Samples were collected by 2 stockworkers per cow house, and each stockworker was required to collect 240 milk samples per day. Stock workers performing this task were video recorded when collecting samples from 5 cows, and the task took approximately 1 minute and 10 seconds to complete. 9 changes in body position were observed in each stock worker;

Figure 8. One of body positions during cow preparation before milking in a traditional type of cowshed – udder hygiene

external load remained below 10 kg. Stock workers performing this task needed to collect samples on a tray and add a preparation to each sample to determine if the milk was fit for consumption. It was found that 1/5 of the time devoted to this task was performed in harmful body positions assigned to Category 3 and 4, since the lumbar spine was simultaneously bent forward and twisted (similarly to the previous task), and stockworkers were forced to squat or kneel.

Milking in a traditional dairy cowshed

This elementary task consisted in cow milking in a traditional cowshed (240 dairy cows). Each stock worker tended to approximately 60 cows, and was required to milk about 120 cows each working day. This work task comprised the following activities: connecting the milking apparatus to the vacuum and milk pipelines, milking, closing each teat with an infection prevention agent, removing the milking apparatus and relocating it to the adjacent stall. Stock workers were video recorded milking a single cow. It took 1 minute and 42 seconds on average to complete the entire milking procedure. During this time, 14 changes in the position of body parts were observed. External load during the milking procedure did not exceed 10 kg as the milk was transported via milk pipe directly to containers. In dairy cowsheds – mainly smaller ones – the milk is collected to containers that are later removed and emptied to the main container. Stock workers are then required to carry significant weights (as high as 20–25 kg). This was not the case in the analysed cowshed.

Milking is another elementary work task in a traditional cowshed, which requires the stock workers to squat or kneel, and to simultaneously bend forward and twist the trunk, which involves considerable static load. These body positions accounted for approximately 20% of the total time devoted to this particular task. Moreover, another 20% of the time was spent with the trunk bent forward and twisted. The highest strain was put on the lower extremities when bending legs or changing body position to squating (when connecting and disconnecting the milking apparatus and when closing the teats). The milking process itself was when the static load on the musculoskeletal system was reduced and stock workers remained in a standing position when the milking process was proceeding for several dozens of seconds.

*Milking in a new dairy cowshed (**Figure 9**)*

This elementary work task consisted in performing a milking procedure in a milking parlour in a new type

Figure 9. One of body positions during cow preparation before milking in a modern type of cow shed

of dairy cowshed. The parlour is equipped with 30 milking stalls in two rows, with 15 cows each. Animals are standing next to each other, turned backwards to the passage. Teats are located at the level of sight of the stock workers, who attach the milking equipment to the teats. Stock workers are no longer required to squat or kneel and the highest static loads typical for traditional cowsheds are eliminated. In the analysed farm, the milking passage was worked by 2 stock workers simultaneously. The stock workers were equipped with 3 milking units, which they carried from one side of the milking passage to another. This saved time, as several cows were milked simultaneously. The activities included in this task were similar to the milking operations performed in a traditional cowshed. Apart from connecting, disconnecting and handling the milking equipment, stock workers also covered the teats with an infection prevention agent.

The working cycle lasted 34 seconds per one cow on average. Within 34 seconds, stock workers changed their body position as many as 38 times. Similarly to milking cows in a traditional cowshed, stock workers were also exposed to external loads, as they had to carry the milking units that weighted less than 10 kg.

The static load was significantly reduced as the squating and kneeling positions were eliminated. None of the body positions was assigned to Category 3 or 4. Moreover, stock workers spent half of the milking time in body positions qualified to Category 1, which were safe and required no corrective measures.

With reference to specific body parts, it was difficult to point out any parts of the body, which were particularly exposed to loads. The trunk was bent for 38% of the working time. In case of upper extremities – one arm was raised above the acronym. Lower extremities were exposed to a slight static load, as the stockworkers were standing on straight legs. Positions with slightly bent legs and squatting were eliminated.

Stock workers habitually assumed a variety of inappropriate body positions in order to be more 'comfortable' at work, which means they were unaware of the associated risks. Inappropriate body postures typically included:

- driving a tractor when removing manure with the trunk bent forward – stock workers should sit straight and lean against the seat,
- turning the head while driving a tractor instead of using rear-view mirrors, which is strenuous for the trunk and cervical spine,
- resting the weight of the body on a single leg only under an additional external load – when removing manure with a fork,
- bending of lower extremities when loading (too much load is carried on tools, legs support the arms, as during manual sweeping of feed),
- moving the milking unit in a traditional cowshed with one arm only held above the acromion.

Discussion

In 6 out of 9 of the analysed elementary work tasks, high and very high static load were identified. The assessment of loads put on particular body parts during work in a traditional dairy cowshed proved what has already been known – the high peak static load is placed on the trunk when simultaneously bent and twisted [23, 24]. Lower extremities are also exposed to significant strain, since stock workers need to squat or kneel when they perform their work on single-level working stands. To maintain balance, stock workers need to rest their hand against the animal and raise their arms above the acromion. This is when the shoulder joint is particularly strained. The outcomes of this study confirm the results of previous alarming analyses presented by other researchers concerning static spinal loads [23, 45]. Moreover, this study confirms the conclusions of previous analyses, which showed that milking in the traditional tethering system was associated with higher peak load for the forearm and biceps muscles than milking in the modern systems [32, 38]. The problem consists of the majority of hazardous body postures being forced body positions. Workers are forced to assume specific body postures during work as the buildings, rooms and appliances for livestock rearing have been designed without any consideration for basic ergonomics [23]. This is particularly evident when the milking methods are compared in traditional and modern dairy cowsheds. In modern livestock rearing facilities, stock workers are not forced to squat or knee, bent forward or hold their trunk twisted, and are exposed to slight static loads only; the same task performed in a traditional cowshed involves significant static loads for over 1/4 of the time spent on performing specific work tasks, and the body position of stock workers requires prompt corrective measures [23, 45].

Key corrective measures:

- Manual removal of manure – replace manual manure removal with a mechanical system; stock workers need to be informed of the risk associated with assuming incorrect body position.
- Cleaning of cow stalls – stock workers need to be informed of the necessity to keep their body straight and to adjust the wash pipe length of the washer to the height and arm's reach of individual stock workers.
- Spreading of litter – introduce specific mechanisms with straw shredder and introduce new habits among stock workers to limit static loads. Workers should avoid bending their legs during work. When the working time with bent legs exceeds 30% of the total working time, the musculoskeletal system is severely affected and corrective measures should be promptly introduced [23, 45].
- Manual feed sweeping – note that the feed passage dimensions at the evaluated workstation allow for the manual feed reloading to be supplemented with a mechanical sweeper (such as a scraper fixed to the front of the tractor or a horizontal sweep auger).
- Udder hygiene and milk sampling – both tasks involve forced body postures. This may be attributed to the design of the workstations, as the stock workers are forced to assume specific body positions to collect a milk sample on a tray, etc. The only solution is to use modern milking methods [2].

The outcome of this study suggests that there is a serious deficiency in the knowledge of basic ergonomic principles for performing physical work among stock workers. Suitable training and the use of kinetic-therapeutic methods may contribute to the limiting the consequences of unergonomic postural loads [3, 11, 28, 33].

Note that a specific share of work in Polish stock farms is performed by women. The consequences of unergonomic positions are particularly noticeable in women [5, 39].

To conclude, the OWAS method may be considered a valuable tool in assessing the static load in dairy cow breeding. However, changeable daily and weekly working times devoted to elementary work tasks may be problematic, as it may be difficult to assess the real risk resulting from such loads on the musculoskeletal system.

Conclusions

1. The elementary work tasks in dairy cow breeding may involve significant loads on the musculoskeletal system. Unergonomic performance of these tasks results from bad habits and the level of mechanisation specific to a dairy cowshed. Consequently, stock workers are forced to assume more or less ergonomic body positions.
2. The proposed corrective and preventive measures presented in the analysis of specific works consists mainly in substituting the tools used so far with more ergonomic equipment, which is safer for the human locomotion system. The implementation of the proposed solutions requires specific investments; however, the risk of locomotor system disorders can be significantly reduced.
3. Specific works, especially in traditional cowsheds, such as cow preparation for milking and the milking process itself, require prompt corrective measures, however, the lack of space may seriously limit the possibility to implement such measures, and stock workers are forced to assume awkward body positions.
4. Education of stock farm staff should become one of the key preventive measures. Educational campaigns should be introduced within the framework of obligatory occupational safety training, in particular. However, the access to occupational safety training among individual farmers in Poland is currently very limited and may pose a challenge.
5. A variety of activities in dairy cow breeding in Poland are performed by women. The consequences of working in unergonomic positions caused by insufficient education and poor technical equipment may be particularly noticeable in women.
6. Further detailed analyses are recommended concerning the loads on the locomotor system and the time devoted to specific tasks, in order to evaluate the actual risk of locomotive disorders in a variety of working circumstances and working conditions in the sector of dairy cow breeding, considering the specific conditions prevailing on stock farms in Poland.

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Conflict of interest statement
The authors declare no conflict of interest.

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Correspondence address:
Bartosz Bilski MD, PhD
Department of Preventive Medicine
University of Medical Sciences
11 Smoluchowskiego St, 60-179 Poznań, Poland
phone/fax: +48618612243
email: bilski@ump.edu.pl

REVIEW PAPER

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Challenges of rehabilitation for patients with primary malignant glioma – a review of recent literature

Katarzyna Hojan[1, 2]

[1] Department of Rehabilitation, the Greater Poland Cancer Centre, Poznan, Poland;
[2] Neurorehabilitation Ward, Bonifratres Rehabilitation Centre, Piaski, Poland

ABSTRACT

Primary malignant glioma is one of the greatest challenges in contemporary rehabilitation. Due to the introduction of newer and newer methods of oncological treatment the overall survival in this group of patients retrospectively increased. This article is a review of the scientific literature of recent years concerning the principles of clinical assessment of patients with primary glioblastoma and the selection of methods in rehabilitation programs based on general condition. In the last years, there has been an increase in the results of clinical studies on the implementation of rehabilitation treatment in primary malignant glioma patients after oncological treatment. Thus, identification of accurate functional performance, behavior changes, evaluation of cognitive function, and cancer markers and oncological treatment as well as quality of life outcomes in this population are of major clinical importance. Primary malignant glioma patients represent a unique patient population with distinctive functional impairments and limitations to physical exercise. Therefore, individual comprehensive rehabilitation treatment may be useful in improving the physical and cognitive functioning as well as decreasing the fatigue syndrome. Such a therapy allows this group of patients to participate in society despite the consequences of cancer treatment.

Keywords: brain tumor, exercise, oncology.

Introduction

Malignant glioma (glioblastoma) is a major challenge in the oncology setting, with median survival nearly 100% of glioblastomas recur, usually within 6–8 months. However the median survival duration of glioblastoma patients in the last years was 14.9 months [1]. Several factors, including age, performance status, tumor grade and histology, and the number of prior progressions, molecular genetic factors, and therapy administered are strong independent predictors of survival in this population [2,3]. The current standard treatment for glioblastoma is surgical resection followed by 6 weeks of conventional fractioned radiotherapy or/and chemotherapy, followed by 12 months of adjuvant chemotherapy [4], although the use of off-label anti-angiogenic agents and other targeted therapies is not uncommon [5]. In a clinical trial setting, the current standard of care for patients with newly diagnosed glioblastoma multiforme (radiotherapy plus temozolomide followed by 6 cycles of adjuvant temozolomide) provided 2- and 5-year survival rates of 27% and 10% [6]. An additional treatment mainstay is the use of high-dose corticosteroids to control intracranial oedema. The use of such aggressive combination therapy together with tumour-related impairments can simultaneously directly (i.e., direct cytotoxic injury) or indirectly (i.e., effects secondary to therapy such as physical inactivity) deleteriously impact the organ components (i.e., the pulmo-

nary-cardiac-muscle axis) that govern exercise tolerance [6]. Poor exercise tolerance leads to a vicious downward cycle characterized by deconditioning (physical inactivity), fatigue, and other functional limitations e.g. body composition changes, muscle atrophy, quality of life (QoL), and depression. Glioblastoma patients represent a unique patient population with distinctive functional impairments and limitations to physical exercise and they have been one of the greatest challenges for rehabilitation in the last years.

Therefore this paper is a review of recent scientific literature concerning the principles of functional assessment and the selection of methods in rehabilitation programs based on general condition in primary malignant glioma patients across the cancer trajectory.

Clinical assessment

Performance status assessment

Physical functioning plays an integral role in modulation of treatment and disease pathophysiology in malignant glioma [2,6,7]. In the current clinical practice, oncologists rely exclusively on the use of subjective performance status scoring systems (e.g., Karnofsky Performance Status (KPS), and the Modified Barthel Index (MBI) or Eastern Cooperative Oncology Group (ECOG) to evaluate functional status in primary malignant glioma patients [8,9]. Study findings show [8–10] that higher KPS and ECOG correlate with improved outcomes. The size, location, and infiltration of a malignant brain tumor may impair the autonomic nervous system response causing dysregulated peripheral sympathetic activation which, in turn, leads to decreased skeletal muscle blood flow and early acidosis. Several methods are available to clinicians that provide objective determinations of physical functioning in the oncology setting [7]. Of these, a 6-minute walk test (6MWT) is a simple and clinically feasible method to evaluate functional capacity and is a robust predictor of mortality in numerous clinical settings [6]. According to the Jones study [11] in 171 patients (70% were diagnosed with glioblastoma multiforme – WHO grade IV, and 85% were undergoing therapy), the 6MWT distance is a clinically feasible tool that provides an objective measure of physical functioning in selection of patients with recurrent glioma. Opposite results of the Ruden et al. study [12] indicate that the clinical utility of the 6MWT may not extend to glioma. A potential explanation is that patients with recurrent glioma may display neurologic impairment that limits their ability to adequately perform a walking test. Other important finding was that functional capacity, as measured by a 6MWT, was not associated with survival in patients with recurrent glioma [12]. On the other hand, Activity Daily Livings scales (ADLs) and exercise behavior are often considered to be synonymous. However, these measures evaluate different aspects of physical functioning [13]. ADLs evaluate the patient's ability to bathe, feed etc., whereas exercise is defined as a planned, structured, and repetitive physical activity performed in leisure time. Together, these results indicate that basic ADLs appear to be well preserved in patients with malignant glioma and, as a result, do not provide prognostic value, whereas exercise behavior successfully discriminates mortality risk. The functional independence measurement and functional activity measurement system (Functional Independence Measures – FIM and Functional Assessment Measures – FAM) may be used to objectively determine impairments in different domains [14]. Therefore, they are recommended for assessment in rehabilitation of glioma patients.

Cognitive dysfunction

Neuro-cognitive function is a very important determinant of QoL. It is well known that impairment of neuro-cognitive functioning, resulting in behavioral, emotional, and intellectual difficulties, occurs in nearly all patients with brain tumors and eventually compromises their independence. The medical factors and complications, including endocrine dysfunction, metabolic disturbances, infection, and pain can also contribute to cognitive and neuro-behavioural changes in this group of patients [15–18]. Psychological reaction such as anxiety, depression, and uncertainty about the future, and a combination of these factors is likely to contribute to cognitive impairment [15]. This impairment is related to a combination of various factors, including the tumor itself, tumor-related epilepsy, oncological treatment, and patient-related factors (e.g., age, psychological distress). Most studies on neuro-cognitive function in brain tumor patients pertain to those with low-grade glioma, and only a few studies have collected follow-up data in high-grade glioma patients [15, 17]. The published studies have generally used a retrospective design, or insensitive screening instruments for this patient population, such as the Mini-Mental State Examination [17]. Cognitive functioning was very often assessed by researchers and clinicians with a battery of standardized tests [19]. Meyers et al. [15] reported that cognitive function but not ADLs was an independent predictor of survival in patients with glioblastoma after adjustment for age, KPS, histology or time since diag-

nosis. Cognitive deficits, potentially compromising QoL, are commonly observed in glioblastoma patients in different stages of the disease [16, 20]. The neuro-cognitive deficit occurs during the oncological disease and so treatment is very important to patients and their caregivers, because these limitations interfere with QoL.

Quality of Life

As Dietz states, in fact, the goal of rehabilitation for people with cancer is to improve the QoL for maximum productivity with minimum dependence, regardless of life expectancy [20]. Porter's study results [21] showed that primary site was significantly associated with functional well-being. Shorter length of time from diagnosis to survey had a significant positive effect on several QoL domains and shorter length of time from completion of radiation to survey was associated with better physical well-being in glioma patients [21]. Common questionnaires, e.g., European Organization for Research and Treatment of Cancer (EORTC) Core Quality of Life Questionnaire, and Functional Assessment of Cancer Therapy (FACT) cancer-specific scales are used to assess health-related QoL in glioblastoma patients [22]. Interpretation of the impact of standard and new therapies on QoL in glioblastoma patients is consequently problematic, even when attempting to classify their effect into the three broad categories of negative, positive, or neutral. In agreement with some brain tumor studies [21, 23], but contradictory to another study [24], were findings of no association between QoL and lateralization of the tumor (left, right, or midline symmetry). The analysis of QoL data is challenging due to the high rates of non-random missing QoL values that may be linked to patients' QoL status, and if ignored may introduce bias in the interpretation of results [25]. Conversely, radiotherapy may decrease QoL in some patients due to adverse effects such as fatigue, somnolence, or cognitive problems. The effects of antiepileptic medication on QoL have been less extensively studied in patients with high-grade glioma, although some studies have reported a negative impact [26]. The effects of corticosteroid would be expected to decrease QoL [27]. Among newly diagnosed glioblastoma patients randomized to radiotherapy alone or radiotherapy plus temozolomide, the addition of temozolomide had no significant negative effect on QoL measures, except on social functioning ($p > 0.05$) [27]. Similarly, among first-relapse glioblastoma patients, temozolomide had no significant negative effect on QoL, although responders to temozolomide had improvement in most QoL scores, e.g., global, motor dysfunction, emotional function, future uncertainty, and communication deficit [28]. However, reliable serial measurement of QoL in patients with primary glioma patients is notoriously difficult, relating to many factors but particularly dropout bias or inability to repeatedly complete complex forms. It would appear that there is a progressive decrease in QoL during the course of high-grade glioma that substantially accelerates once the disease relapses. This is also expressed as deterioration peaks driven by the therapies administered (e.g., radiotherapy) or by the exacerbation of accompanying syndromes (e.g., brain edema, neurological symptoms, psychiatric disturbances).

Fatigue syndrome

Cancer-related fatigue is defined by the National Comprehensive Cancer Network (NCCN) as a persistent, distressing, subjective sense of physical, emotional, and/or cognitive tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with usual functioning [29]. The average level of fatigue experienced by glioma patients is about 40–50% higher than normative levels for cancer patients, equating to approximately five times the clinically meaningful difference [7, 30, 31]. Powell with collegues' study [32] demonstrated that fatigue is a prominent pretreatment symptom in patients with newly diagnosed and operated glioblastoma, reaching a prevalence of 48% compared with only 11% among healthy controls. Fatigue in patients with primary brain tumors has repeatedly been reported in relation to radiotherapy [32]. The authors indicated that the contribution of toxicity from radio-chemotherapy to fatigue is probably only one factor among many. After Peters' study [33] the authors concluded that greater degree of fatigue was associated with poorer survival in high-degree glioma patients, and FACT scales are not independent predictors of prognosis. Fatigue was a strong independent predictor of survival that provides incremental prognostic value to the traditional markers of prognosis in recurrent glioma [33]. The authors concluded that pharmacological or non-pharmacological strategies (e.g. rehabilitation) are effective methods to decrease the fatigue syndrome.

Practical rehabilitation

Physical exercises

Increasing evidence suggests that exercise modulates a range of systemic factors (e.g., metabolic and

sex-steroid hormone concentrations, immune surveillance/cytokine or angiogenic factors, and products of oxidation) that, in turn, may alter ligand availability in the tumor microenvironment with subsequent effects on relevant cell signaling pathways [34, 35]. Markedly reduced strength and fitness capabilities compared to age- and sex-matched norms have also been reported in glioblastoma patients [36]. For example, the maximal muscular strength was observed to be 57 ± 28% of predicted values and cardiorespiratory fitness reported to be 41 ± 10% of predicted values among clinically stable patients following surgery and unfavorable changes in body composition are also apparent with a loss of lean mass and gains in fat mass evident following surgery [36]. Many randomized trials demonstrate that structured physical training is a safe and well-tolerated therapy associated with significant improvements in several clinically relevant outcomes, such as cardiorespiratory fitness, QoL, and fatigue in patients with other cancer than brain tumors both during and after primary adjuvant therapy [34]. Actually, there have been no randomised clinical trials evaluating the efficacy of exercise in counteracting the physical impairments experienced by primary glioma patients. In the study by Schmitz et al. [37], an unexpected finding was the relatively high number of participants who reported meeting the American College of Sports Medicine exercise prescription guidelines for cancer survivors of achieving at least 150 min per week of strenuous/moderate exercise [38]. Physical exercise may represent a supportive intervention that may complement existing neuro-oncologic therapies and address a multitude of therapy-induced debilitating side effects in patients with brain tumours. In recent years, increased attention has focused on exercise as a rehabilitative intervention for cancer survivors both during and after the cessation of cancer therapy [34,39]. In the study by Hansen [40], the authors prepared exercise training for glioma patients which included individually tailored strength training of main muscle groups with increasing load ranging from 15 to 10 repetition maximum (RM) (leg press, arm flexion, arm extension, knee flexion and knee extension), cardio-training (20 min of cycling or treadmill with intensities ranging from 65% to 85% of the heart rate reserve), body awareness training or relaxation (training of proprioception, postural control or stability of the core muscles tailored to personal needs). The strength training workload was calculated based on baseline tests and included in patients' training diaries with progression instructions. The cardiovascular training was monitored by pulse by means of a wireless heart rate transmitter worn by the patients [38]. The authors [40] did not observe any side effects of this training during 6 weeks. Physical exercise in glioma patients may trigger processes facilitating neuroplasticity and, thereby, enhances an individual's capacity to respond to new demands with behavioral adaptations. A final and important potential mechanism is an abnormal neurohormonal response to exercise due to disease burden and surgical excision of normal brain tissue. The exercise response is governed by the interplay between central command and afferent information from the exercising muscles [40].

Neuropsychological training

Cognitive impairment is one of the most common neurological disorders in brain cancer patients and exerts a deep negative impact on QoL interfering with family social and career-related activities. It is well known that oncological treatment may increase cognitive deficits. For example anaemia and fatigue, common symptoms in patients with glioblastoma, might affect cognitive function. Massa and et al. [41] investigated the effectiveness of erythropoietin during chemotherapy on cognitive function in ten elderly patients with cancer and anaemia, and their results supported the hypothesis that increases in haemoglobin concentrations are accompanied by significant improvement in cognitive performance as measured by the Mini-Mental State Examination. Pharmacologic interventions have not proven effective yet in the treatment of cognitive deficits in patients with glioblastoma. Cognitive rehabilitation interventions represent an alternative treatment approach. Zucchella et al. [42] in randomized controlled trial of cognitive training for glioma patients demonstrated a significant enhancement of cognitive performances after the 16 one-hour individual session of cognitive training (combining computer exercises and meta-cognitive training). In rehabilitation group the authors showed [42] a significant improvement of cognitive functions especially the visual attention and verbal memory. In a randomised controlled trial in 140 adult patients with low-grade and anaplastic gliomas after cognitive rehabilitation (individual two-hour sessions six times a week; conducted by one of neuropsychologists, incorporating both cognitive retraining and compensation training) Gehring with collegues [43] observed significant improvement in self-reported cognitive functioning at the immediate post-intervention assessment, and during the 6-month follow-up assessment – significantly better results than the control group tests of attention and verbal memory. The

patients also reported less mental fatigue. The intervention incorporated both computer-based attention retraining and compensatory skills training of attention, memory, and executive functioning [43]. Alvares with colleagues [44] suggest that EEG biofeedback has potential for reducing the negative cognitive and emotional sequelae of cancer treatment as well as improving fatigue and sleep patterns. New evidence indicates that exercise exerts its effects on cognition by affecting molecular events related to the management of energy metabolism and synaptic plasticity [45]. Physical exercise has demonstrated an extraordinary aptitude to influence molecular pathways involved in synaptic function underlying learning and memory. An instigator in the molecular machinery stimulated by exercise is brain-derived neurotrophic factor, which has an influence on the interface of metabolism and plasticity [45].

Occupational therapy

Occupational therapy is a very important element of comprehensive rehabilitation. It comprises training in activities of daily living such as bathing, grooming, dressing, toileting, meal preparation, and homemaking. It is one of the most important exercises during oncological treatment [46]. In addition, occupational therapists evaluate home environments for potential modification, provide instruction in driving with adaptive devices, and implement interventions to promote upper extremity range of motion (ROM), strength, endurance, and coordination. The training focuses on bettering the patients' functional capacity, body, activity and participation level by adapting activities, regaining or developing activity abilities and/or rebuilding and developing patient skills [38]. In the study by Yoon et al. [45] conducted in 40 patients, the authors concluded that virtual reality-based rehabilitation combined with conventional occupational therapy may be more effective than conventional occupational therapy, especially for proximal upper-extremity function in patients with brain tumor.

Discussion

Because of the recent advances in surgical techniques, chemotherapy, and radiation therapy, survival times of patients with glioblastoma have increased and more of these patients require rehabilitation support and services [47, 48]. The International Classification of Functioning, Disability and Health (ICF) [49] framework defines a common language for describing the impact of disease at different levels. For example, brain tumour related 'impairments' (headaches, seizures, neuro-cognitive dysfunction, paresis, dysphasia), can limit 'activity' (decreased mobility, inability to self-care) and 'participation' (work, family, social reintegration), and reduce QoL [50]. FIM-FAM system is relatively simple, easy to perform in routine clinical practice and may be used as a tool for assessment of rehabilitation programs, especially in neurological disorders [14]. A close relationship between the medical and rehabilitation teams is necessary to maximize improvement because rehabilitation can be hampered by treatment side effects. It is plausible to assume that neuro-cognitive function, irrespective of clinical stage, may also have prognostic implications even after initiation of therapy and during the course of oncological treatment. Few studies have addressed the problem of methodology in glioblastoma patients in depth, and several limitations have to be mentioned. First, many groups have included patients with all sorts of primary brain tumors despite large differences in underlying neurobiology, treatment procedures, and prognosis. The majority of studies have reported statistically significant findings across a wide range of psychosocial (e.g., depression, anxiety, symptoms, etc.) and physiologic (e.g., muscle strength, immune and metabolic profiles, body composition) endpoints, culminating in clinically meaningful improvements in the patient's functional capacity and overall QoL in cancer patients [51, 52]. Many recent trials recommend comprehensive rehabilitation intervention in primary glioma patients in all stages of the disease for restoring function after cancer therapy, and in advanced stages of the disease as important part of palliative care with the aim to prevent complications, control the symptoms and maintain patients' independence and QoL [45, 47, 53–55].

To conclude, primary malignant glioma is one of the greatest challenges in contemporary rehabilitation. Identification of accurate functional performance, behaviour changes, evaluation of cognitive function, and markers of prognosis in oncological treatment may be useful in implementing individual comprehensive rehabilitation treatment. Such a therapy allows this group of patients to participate in society despite the consequences of cancer and oncological treatment.

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Correspondence address:
Katarzyna Hojan MD, PhD
Department of Rehabilitation
The Greater Poland Cancer Centre
15 Garbary St, 61-866 Poznan, Poland
phone: +48618850705
fax: +48618521948
e-mail: khojan@op.pl

REVIEW PAPER

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Primary aldosteronism as an endocrinological challenge – old doubts and new diagnostic possibilities

Ewa Cyrańska-Chyrek[1], Małgorzata Grzymisławska[2], Marek Ruchała[1]

[1] Department of Endocrinology, Metabolism and Internal Medicine, Poznan University of Medical Sciences, Poland
[2] Department of Anatomy, Poznan University of Medical Sciences, Poland

ABSTRACT

Hypertension constitutes a common clinical problem worldwide. In fact, a systematic increase in its detection is predicted in the following years, with early detection, accurate diagnosis and effective treatment of hypertension being a priority. The most common endocrinological cause of hypertension is primary aldosteronism. What is more, elevated aldosterone levels cause a deterioration in blood pressure normalization, diabetes, and significantly increase cardiovascular risk. There are two distinct causes of primary aldosteronism – aldosterone producing adenoma (APA), as well as bilateral adrenal hyperplasia (BAH) and proper differentiation between APA and BAH has clinical implications. In the case of the former adrenalectomy is advised, whereas the latter is followed by introduction of proper pharmacotherapy with aldosterone antagonists (spironolactone, eplerenone).

Keywords: primary aldosteronism, adrenal tumor, aldosterone producing adenoma, bilateral adrenal hyperplasia, adrenal CT, adrenal venous sampling.

Introduction – epidemiology of hypertension

Hypertension is a common, serious clinical problem which currently affects about 1 billion patients worldwide. Global prognoses indicate further systematic increase in the affected population exceeding 1.56 billion in 2025 [1]. Furthermore, hypertension constitutes a challenge for health care all over the world and its early detection and effective treatment are a priority.

The majority of cases, about 90–95%, are described as primary hypertension, therefore it is not possible to precisely determine its etiology. Hence, the unfortunate name of idiopathic hypertension. On the other hand, over 40 causes of secondary hypertension have been identified up to date, including ones with an endocrinological background, the most common being hypertension with primary aldosteronism, but also others such as hyper- and hypothyroidism, hypercortisolemia, hyperparathyroidism or pheochromocytoma [3].

Taking into consideration the fact that 20–30% of patients are diagnosed with resistant hypertension, it seems obvious and necessary to seek for the underlying cause. This in turn provides a chance for an individualized patient approach and allows for effective treatment [4].

Clinical manifestations and etiopathogenesis of primary aldosteronism

Primary aldosteronism (PA) is now considered to be the most common and potentially reversible secondary cause of hypertension which causes about 5 – 13% cases of hypertension [5–7]. It is estimated that an excess of aldosterone occurs in 5 to 40% cases of

hypertension [8–10]. According to some authors, this phenomenon can no longer be called an epidemic [11–13] and, on the contrary Galati SJ et al. suggest that many patients still remain undiagnosed [14].

In the literature, primary aldosteronism (PA) is also referred to as Conn syndrome [15]. It is clinically manifested by fatigue and periodic skeletal muscles weakness, hipokaliemia and metabolic alkalosis. The description of the syndrome from 1955, is frequently considered to be the first published one, although 2 years prior to its publication, two cases of PA hypertension were presented by a Polish practitioner Michał Lityński [16].

Until recently, the coincidence of adrenal tumor with resistant hypertension and spontaneous hypokalemia were treated as clear indication of primary aldosteronism. However, in the past 60 years since PA was first described, a number of discoveries have been made which frequently contradict the original assumptions related to the condition.

Diagnostic difficulties in primary aldosteronism differentiation

It is currently believed that the most common causes of PA are bilateral adrenal hyperplasia (BAH), which constitutes about 60% of cases, and aldosterone producing adenoma (APA), aka. aldosteronoma, which adds up to 40%. What is interesting, until recently the ratio had been believed to be quite the opposite, with APA causing two-thirds of PA cases [17]. However, scientific advancement has facilitated a breakthrough in determining PA etiopathogenesis.

Interestingly, with the increased availability of imaging techniques (especially computed tomography, CT) numerous doubts have arisen concerning the differentiation between the causes of PA. They point to the fact that adrenal adenoma imaging in a patient with hypertension and hypokalemia is not decisive in aldosteronoma diagnosis. In fact, there is a possibility of imaging a non-functioning adenoma (NFA) instead of a real underlying cause, i.e. a contralaterally located microadenoma, which was not revealed in the CT [18].

Young WF et al. analyzed 203 PA patients. They showed that proper differentiation between APA and BAH was performed only in 53% of cases. As a result of biochemical and imaging analysis, 20% of patients would be incorrectly disqualified from adrenalectomy, whereas in another 25% the surgery type would be improper. Diagnostic difficulties are further enhanced by the fact that aldosteronomas are frequently microadenomas (with a diameter < 1 cm); thus, failure to present them in the CT with concomitant clinical hyperaldosteronism manifestations may incorrectly imply hyperaldosteronism [19].

Reliable diagnosis associated with definite lateralization of hormonally active lesions has been possible since 1967, when adrenal venous sampling (AVS) was introduced. Unfortunately, it is a difficult and invasive method, especially due to the anatomical limitations (mainly small size of the right suprarenal vein) and the catheterization of both veins in order to compare the results. Moreover, it is available only in very few medical centers. The Mayo Clinic experience shows that AVS procedures allow for an accurate diagnosis in 95.5% of cases, whereas diagnosis based exclusively on the biochemical and imaging tumor assessment is correct only in 58.6% of patients [20].

The authors point out that suprisingly only 25% of the Mayo Clinic patients consents to AVS; the remaining 75% choose pharmacotherapy. Artl W. et al. additionally stress that even in the most advanced centers, the percentage of successful AVS procedures is not higher than 40–70%, mainly because of anatomical limitations in the course of procedure [21].

Variety of clinical manifestations in primary aldosteronism

Research conducted in recent years has shown that PA is also present in normotensive patients [22–25], as well as those with mild to moderate hypertension without concomitant hypokalemia. The Italian researchers' study on a large number of 1125 patients indicate that a large percentage of patients shows PA independent of normokalemia. In fact, hypokalemia was diagnosed in 48% of APA patients and 16.9% of idiopathic hyperaldosteronism patients (IHA).

Furthermore, aldosteronoma is diagnosed more frequently in centers where AVS is available. In such cases the APA to IHA ratio is 62.5% to 37.5% respectively. On the other hand, in the centers where AVS is not accessible the APA to IHA ratio is 35% to 65% respectively [27].

Numerous researchers stress the incidence of primary aldosteronism in hypertensive patients without concomitant hypokalemia. According to some authors, it points to the necessity of widening PA screening to both normokalemic and normotensive patients [28–30]. On the other hand, Kaplan NM points out

that the moderate hypertension (with RR values of 160–180 mmHg/100–110 mmHg) includes up to 25% of hypertensive patients, whereas an adrenal incidentaloma is misdiagnosed for aldosteronoma in only 1% of cases of adrenal incidentaloma [31, 32].

Adrenal incidentalomas (AI) have become another fundamental issue. According to the literature, PA is diagnosed in adrenal incidentaloma patients in 1.6–3% of cases [33, 34]. However, due to many limitations and difficulty in maintaining conditions necessary for proper diagnosis of aldosteronemia, as well as doubts concerning proper cut-off point in the aldosterone to renin ratio (ARR), the influence of various drug groups and clinical states on the ARR interpretation must be taken into account [35]. Moreover, in women both aldosterone and ARO levels strictly correlate with the phase of the menstrual cycle which induces elevated aldosterone level (> 15 ng/ml), as well as PA overdiagnosis. It is the case in 30% of female patients in the 7th day of cycle, and it increases even to 70% in the 21st day [36, 37]. Ahmed AH et al. suggest the need of establishing separate norms and cut-off points not only for men and women, but also for individual menstrual cycle phases [38].

Hyperaldosteronism's clinical implications

The diagnosis and effective treatment of PA is even more important in terms of increased occurrence of cardiovascular incidents, strokes and arrhythmias in PA patients. As it turns out, PA patients are more at risk of a myocardial infarct than those suffering from essential hypertension (EH), with the PA to EH ratio of 20% to 8% respectively. They are also more prone to sustain a stroke or transient ischemic attack (11% PA vs. 3% EH), to have arrhythmias (15% PA vs. 3% EH), as well as to suffer from chronic lower limb ischemia (65% PA vs. 2% EH) [39].

Numerous analyses indicate a more common incidence of pre-diabetic states and type 2 diabetes in PA patients than in cases of obese and/or with essential hypertension patients [40, 41, 42]. Modern medical knowledge clearly states that undiagnosed or ineffectually treated PA definitely deteriorates maintaining sugar levels and increases albuminuria which constitutes an independent cardiovascular risk factor [43, 44]. Many clinical research points out to adverse metabolic profile observed in PA patients. In fact, they have shown a lower adiponectin concentration, higher resistin and leptin levels, as well as increased insulin resistance [42, 45–47]. Additionally, in comparison to essential hypertension patients, PA patients run the risk of accelerated development of cardiac remodeling, i.e. thickening and increased mass of the left ventricle muscle [48, 49]. The above mentioned conclusions may partially account for an increased cardiometabolic risk in comparison with essential hypertension (ES), as well as higher mortality rates observed in these patients [50].

Aldosterone activates mineralocorticoid receptors (MR) found in the heart, blood vessels and the brain. Additionally, the biological effect of their stimulation are heart and blood vessels fibrosis, pro-arrhythmogenic and pro-inflammatory action, as well as vascular endothelium damage which, as a consequence, increases the risk of cardiovascular system disorders [51].

Vast variety of aldosterone mechanisms

In recent years, the dependence between elevated aldosterone level and hemostatic disorders leading to the increased risk of thromboembolic incidents has been shown. It was observed that aldosterone impairs vascular endothelium function and fibrinolysis, as well as increases oxidative stress. Furthermore, in the experimental arterial thrombosis rat-models it has been proven that long-term aldosterone administration increases the thrombotic process [52]. Pro-thrombotic aldosterone action is complex and depends on the activation of the primary hemostasis, pro-coagulation and antifibrinolytic activity, as well as a decrease in nitric oxide bioavailability and an increase in oxidative stress. What is more, hormone effects were not fully removed after mineralocorticoid receptor blockade thus implying the role of alternative mechanisms in the hormone pro-coagulation activity [53].

It was claimed that aldosterone, similarly to other steroid hormones, acts only through specific cytoplasmic mineralocorticoid receptor MR. In view of recent data, the theory regarding non-genomic (local) aldosterone action has become more relevant. The aforementioned action has been confirmed in many experimental models in various cell types, such as vascular smooth muscle cells, lymphocytes, and endothelial cells [54. 55]. Furthermore, aldosterone has become more important than expected in the cardiovascular system pathology, as a local messenger. Addition-

ally, the majority of experimental data indicate that non-genomic (local) aldosterone activity is visible within a few minutes and is not blocked by traditional MR receptor antagonists (spironolactone, canrenone and eplerenone) [56, 57, 58]. Therefore, the role of new membrane receptors is alleged, as well as alternative routes associated with the activity of potassium ions, angiotensin II or the activation of glucocorticoid receptor [59].

Due to the MR receptor presence in the adipose tissue and the vascular endothelium, earlier administration of mineralocorticoid receptor antagonists is advisable in PA patients [60, 61, 62]. Since primary aldosteronism has multifold and serious consequences, some authors included the MR receptor antagonists as a first line treatment [63[, even in patients with mild and moderate hypertension [64].

In addition, comparative studies are carried out aimed to verify which of the MR receptor antagonists (spironolactone or eplerenone) is more effective. Some authors suggest a better hypotensive effect of spironolactone in PA patients [65], others did not observe any difference in the hypotensive effect [66].

"Lost subtype" of primary hyperaldosteronism

Research conducted by Spath M and Willenberg HS shows that although some adrenal adenomas are classified as aldosteronoma, they actually also overproduce cortisol. Thus, the comorbidity of hyperaldosteronism and subclinical hypercortisolemia is referred to as primary aldosteronism/subclinical Cushing syndrome (PA/SCS). As a consequence, it may make the PA diagnosis difficult, and at the same time cause cortisol deficiency following adrenalectomy. The authors indicate that aldosterone– and cortisol-co-secreting tumor should be suspected in case of any patient with a tumor >2.5cm, partial suppression in dexamethasone suppression test and or increased corticosteroid excretion in urine. The authors refer to such combination of symptoms as the "lost subtype of primary aldosteronism" [67, 69].

The aforementioned problem may affect even 10% of PA patients which indicates SCS screening in every PA case [69]. These observations are confirmed by Hiraishi K et al. They carried out an analysis of 38 patients with PA – in 21% (8/38 patients) they recognized the coexistence of PA / SCS. These patients were older, adrenal tumors were of larger size, presented higher levels of kalemia and lower levels of serum aldosterone. Moreover, six of them require replacement after corticosteroid therapy adrenalectomy [70, 71].

On the other hand, some contradictory opinions have also appeared. In fact, Markou A et al. concluded that elevated aldosterone serum levels may also be observed in hypertensive patients without PA, and they result from glomerular zone increased response to the ACTH excessive stimulation. These patients also benefit from the MR antagonist treatment [72].

Imaging limitations in aldosteronoma

In case of difficult AVS availability, doubtful imaging diagnosis as well as bilateral lesions and/or lack of patient's consent for invasive treatment, scintigraphy with iodomethyl-norcholesterol (NP-59) may be considered aimed at hormonally active lesion lateralization [73]. The analysis of the research conducted between 1979–2003, where the total of 686 patients were included, presented NP-59 scintigraphy sensitivity at the level of 86%, specificity – 78%, and accuracy – 82% [74]. As a result, this method was considered valuable in the surgery qualification [75].

Nevertheless, its limitations should also be considered. Due to the examination's low resolution and the overlapping activity of the liver and/or the intestines, it is not useful in terms of microadenomas and adenomas with the diameter < 1.5 cm. At the same time, it requires special preparation of the patient in order to obtain optimal uptake of radiotracer which includes discontinuation of the angiotensin II receptor blocker (ARB), angiotensin-converting-enzyme inhibitors (ACE-I), as well as diuretics in 4–6 weeks prior to the examination according to a given protocol [76].

New updates

New information concerning a newly discovered rare PA form has recently surfaced in the literature, described as a surgically treatable unilateral adrenal hyperplasia (UAH). Goh BK et al. analyzed 30 described patients suffering from UAH, hypertension, hypokalemia and elements of primary aldosteronism who were successfully treated by means of unilateral adrenalectomy [77].

Moreover, new data have been emerging regarding the association between PA and level of parathormone concentration (PTH). In fact, this phenomenon resembles a vicious circle, i.e. aldosterone increases the parathyroid hormone secretion by binding with mineralocorticoid receptors (MR) found in the parathyroid cells,

whereas PTH directly stimulates aldosterone synthesis in the adrenal cells glomerular zone. PTH increases the risk of cardiovascular damage by binding with PTH receptor (PTHR) present in cardiomyocytes and vascular smooth muscle cells. Additionally, by its pro-inflammatory activity PTH may increase the cardiovascular damage. MR antagonist therapy combined with angiotensin-converting-enzyme inhibitors (ACE-I) suppress the mutual dependence [78, 79].

Zhang LX et al. observed that the measurement of PTH may be vital for aldosteronoma diagnosis. 142 patients with adrenal tumor were qualified for the research and in 84 cases APA was diagnosed, whereas in 58 patients a non-functioning adenoma (NFA) was found. Furthermore, PTH level was significantly increased, while the level of calcium and phosphates was considerably decreased in APA patients as compared to NFA cases. In addition, the tilt test revealed that a change in PTH concentration (Δ PTH) was greater in APA patients than in NFA patients. The author presents an additional auxiliary tool in APA diagnosis, i.e. measuring both PTH at the baseline level and in the tilt test in cases where primary aldosteronism is suspected [80].

Due to a still growing number of NT patients, as well as constant development in data regarding secondary hypertension, including PA, PA itself should be considered each time a hypertensive patient is treated. The correct diagnosis of the underlying cause of hypertension allows for effective treatment or at least improvement. As a consequence, it enhances patient cooperation and results in better long-term treatment effects.

Limitations of the biochemical and imaging examinations in the PA diagnosis may result in the decrease in the incidence of misdiagnoses, both false-positive, and false-negative. Despite a number of doubts, it is necessary and beneficial to make attempts at accurate diagnoses, as well as to initiate adequate treatment. What is more, owing to the analyzes conducted on 5 continents, it is known that the majority of treated patients are cured or achieve clinical improvement, respectively: 55% to 45% (Brisbane), 70% to 30% (Santiago), 65% to 35% (Torino), 33% to 66% (Rochester), 40% to 55% (Singapore) [10]. The aforementioned observations indicate an urgent and inevitable need to optimize the biochemical diagnosis (aldosterone, ARO, PRA), as well as PA imaging. Moreover, due to the visible limitations of the latter, also a wider access to adrenal venous sampling procedure is necessary.

Summary

40 causes of secondary hypertension have been recognized up to date, where primary aldosteronism constitutes the most common reversible cause, present in 5–13% of hypertensive patients.

The most common causes of PA are bilateral adrenal hyperplasia (60% of cases), and aldosterone producing adenoma aka. aldosteronoma (40%). However, imaging an adrenal adenoma during imaging examination in a hypertensive and hypokalemic patient does not correspond to diagnosing aldosteronoma. In fact, it is difficult to make a correct diagnosis due to the increasing number of incidentalomas (the possibility of imaging a non-functioning adenoma), as well as to the possibility of normokalemia and normotension in PA patients. Correct qualification of PA adenoma patients to adrenalectomy eliminates and/ or markedly limits the adverse reactions to increased aldosterone level. Reliable functioning lesion lateralization has been possible since 1967 when adrenal venous sampling was introduced. Nevertheless, due to anatomical limitations and technical difficulties it is available only in very few medical centers.

In addition, the concomitance of increased aldosterone level and hemostatic disorders was described, including vascular endothelium damage, fibrinolysis impairment and increased oxidative stress. As a consequence, it influences the increased occurrence of cardiovascular incidents, cerebral strokes and arrhythmia in PA patients when compared to patients suffering from essential hypertension.

Due to a multifold serious consequences of hyperaldosteronism, sooner administration of mineralocorticoid receptor antagonists in the treatment of PA patients was recommended, even in cases of mild and moderate hypertension. Recent research has pointed to a possible comorbidity of hyperaldosteronism with subclinical hypercortisolemia ("the lost subtype of primary aldosteronism"). It is also referred to as primary aldosteronism/subclinical Cushing syndrome (PA/SCS) which makes the diagnosis difficult, and may be the cause of cortisol deficiency following adrenalectomy.

Numerous attempts at the differentiation of non-functioning adenomas and aldosteronomas are made. The association between PA and increased levels of parathyroid hormone (PTH) was observed. In fact, it resembles a vicious cycle which contributes to earlier damage of the cardiovascular system by means of binding with PTH receptor (PTHR), present in cardiomyocytes and vascular smooth muscle cells. Thera-

py based on MR antagonists and angiotensin-converting-enzyme inhibitors results in the suppression of the mutual dependence.

The limitations of biochemical and imaging examinations imply the necessity of better access to adrenal venous sampling procedure. Proper qualification of aldosteronoma patients following adrenalectomy results in higher percentage of cured patients or achieving clinical improvement.

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Correspondence address:
Department of Endocrinology,
Metabolism and Internal Medicine
University of Medical Sciences
49 Przybyszewskiego St, 60-355 Poznan, Poland

REVIEW PAPER

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Amyloidosis – short review

Agnieszka Gaczkowska, Paweł P. Jagodziński, Adrianna Mostowska

Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poznan, Poland

ABSTRACT

Amyloidosis is a heterogeneous group of disorders associated with pathological deposition of amyloid. We can recognize two major categories of amyloidosis: primary (AL) and secondary (AA) type. Systemic monoclonal immunoglobulin light-chain (AL) is the most common form of systemic amyloidosis. Systemic AA amyloidosis is associated with chronic inflammation or infective diseases and is the second common form of systemic amyloidosis. The golden standard in diagnosis of amyloidosis is biopsy. The model of treatment depends of type of amyloidosis. In some cases cell transplantation can be considered. In AA the purpose is to decrease inflammation.

Keywords: amyloidosis, amyloid, diagnostic, treatment.

The first description of the amyloidosis was probably reported by Nicolaus Fontanus in 1639. The autopsy of a young man showed ascites, jaundice, epistaxis, abscess in the liver and large spleen with stones. The term 'amyloid' was used the first time by a German botanist in 1838. This term means a normal amylaceous constituent of plants. In medicine this term appeared in 1854 and it was involved in the case of nervous system disease described by Rudolph Virchow. The amyloid substituted another terms: 'lardaceous' and 'waxy' changes [6].

Amyloidosis is a heterogeneous group of disorders associated with pathological deposition of protein (extracellular amyloid) in an abnormal fibrillar form. The extracellular amyloid deposits are present also in Alzheimer's, Huntington's and Parkinson's diseases, familial Mediterranean fever or dementia with Lewy bodies. The fibril type is the basis for the classification of amyloidosis. We can recognize two major categories of amyloidosis: primary (AL) and secondary (AA) type. There are other, less popular types, which include hereditary mutant transthyretin (ATTR), dialysis associated (with β_2-microglobulin β2M) disease, age-related (senile) systemic amyloidosis and organ specific amyloidosis (**Table 1**) [1, 3, 7, 8].

Types of amyloidosis

Systemic monoclonal immunoglobulin light-chain (primary type AL) is the most common form of systemic amyloidosis. It is characterized by systemic disease (primary amyloidosis, multiple myeloma, Waldenstrom's lymphoma) and local disease (skin, urinary tract, larynx, eyes). Onset of the disease may result in non-specific symptoms. This may be enlargement of the spleen or liver, edema (proteinuria, hypoalbuminemia, congestive heart failure), peripheral sensory neuropathy, carpal tunnel syndrome, diarrhoea, constipation (autonomic dysfunction), orthostatic hypotension, symptoms of cardiomyopathy (50% of patients), enlarged tongue, in appearance of blood around the eyes, nail dystrophy. In radiographic manifestations of systemic amyloidosis we can identify parenchymal findings: reticular nodular opacities, reticular opacities and diffuse alveolar opacities. Primary systemic amyloidosis can involve the kidneys, gastrointestinal tract, skin, respiratory system, heart, and other organs. However, there are known forms of localized amyloidosis affecting only one organ. The prognosis for patients is bad since this disease is rapidly progressive, affects multiple organs and median survival is 5 months from diagnosis [2, 4,

Type	Symbol	The most common symptoms	Therapy
Primary type	AL	Systemic or local disease: enlargement of the spleen and liver, neuropathy, cardiomyopathy	High-dose alkylator-based chemotherapy, stem cell transplantation, diuresis, serial thoracenteses, pleurodesis, bronchial repermeabilization
Secondary/reactive type	AA	Diseases of urinal system – proteinuria, splenomegaly, functional hyposplenism, amyloid deposits at the adrenal glands, liver and gastrointestinal tract	Immunosuppressants, colchicine, demethyl sulfoxide, immunotherapy
The hereditary types, the most common - transthyretin amyloidosis	ATTR (transthyretin amyloidosis) AFib (fibrinogen Aα chain) ALys (lysozyme) AApo AI (apolipoproteins AI) AApo AII (apolipoproteins AII) AGel (gelsolin)	Peripheral neuropathy, autonomic neuropathy, cardiomyopathy, ophthalmopathy	Early liver transplantation, possibility of hepatorenal or hepatocardiac transplantation
Dialysis-related/β2-microglobulin type	DRA	Carpal tunnel syndrome, bone cysts, destructive arthropathies, spondylarthopathies	Renal transplantation, hemodialysis with high-flux membranes and β2-microglobulin adsorption columns, steroids, non steroidal anti-inflammatory drugs
Senile systemic type	SSA	Heart failure, atrial arythmias and cardiomegaly	Drugs therapy (diuretics, beta blockers, angiotensin enzyme inhibitors, angiotensin receptor blockers, digoxin), ventricular assist devices, heart transplantation

Table 1. Amyloidosis review. The Table is based on articles cited in the text

5, 9]. There are also extremely rare types of amyloidosis: with heavy chains (AH) and heavy and light chains (AHL), both included in the Ig-related amyloidosis. All described cases of AHL had renal involvement, patients are less likely to suffer from cardiac type and there was better patient survival than with AL [12, 19].

Systemic AA amyloidosis (known also as reactive or secondary) is associated with chronic inflammation or infective diseases. It is the second common form of systemic amyloidosis and it is popular in Europe and in developing countries (30–40% of renal cases). Patients with AA are usual younger than those with AL. High risk factors of AA are: arthritis – the common reason, hereditary periodic fevers, inflammatory bowel diseases, chronic infections (e.g. chronic cutaneous ulcers and osteomyelitis), immunodeficiency states, systemic vasculitis, neoplasia, tuberculosis and sarcoidosis. Clinical signs usually involve urinal system, including proteinuria in 95% and nephrotic syndrome in more than 50% of cases. Other common symptoms are massive splenomegaly, functional hyposplenism, amyloid deposits at the adrenal glands, liver and gastrointestinal tract. Cardiac and neuropathic symptoms are extremely uncommon [10–13]. AA type is caused by reactant protein serum amyloid A (SAA). SAA is synthesized under the cytokine control and it can increase in acute injury, infection or inflammation. There are two isoforms of SAA – SAA1 and SAA2. AA can be a serious complication for patients with the hereditary periodic fever syndromes: familial Mediterranean fever, the TRAPS syndrome, Muckle-Wells syndrome, and hyperimmunoglobulinemia IgD with periodic fever syndrome [32–34].

The hereditary amyloidosis is a rare group of disorders. It is mainly inherited as autosomal-dominant disorder. The most common type of familial amyloidosis is transthyretin amyloidosis (ATTR), but we can recognise other types with proteins including fibrinogen Aα chain (AFib), lysozyme (ALys), apolipoproteins AI (AApo AI) and AII (AApo AII), and gelsolin (AGel) [13–16]. ATTR includes five phenotypes: familial amyloid polyneuropathy type I or II, familial amyloid cardiomyopathy, familial oculoleptomeningeal amyloidosis and familial leptomeningeal amyloidosis. It can be presented as peripheral neuropathy, autonomic neuropathy, cardiomyopathy and ophthalmopathy. The amyloid-producing pathway is associated with mutations in the amyloid-β domain. The mutations of Alzheimer amyloid precursor protein are associated with the systemic hereditary amyloidosis [22–31]. To date, more than 90 single nucleotide variants have been identified in the coding sequence of *TTR* (OMIM *176300) in individuals with this type of amyloidosis. Familial ATTR amyloidosis is inherited in an autosomal dominant manner. Prenatal diagnosis is offered to all pregnant women if the pathogenic *TTR* variant has been identified in their families [25].

Dialysis-related amyloidosis (DRA) is caused by deposition of β_2-microglobulin (β2M) in tissues during long-term dialysis in chronic kidney disease patients. The clinical sings include carpal tunnel syndrome – the most characteristic symptom, bone cysts, destructive arthropathies, spondylarthopathies and sometimes involve other organs like skin, liver, heart or spleen. Risk factors of DRA are: age, duration of dialysis, use of low-flux dialysis membrane and genetic factors, e.g. apolipoprotein alleles, which are correlated with Alzheimer disease. The genetic risk is the highest in the case of mutation in apolipoprotein ε4 allele [17, 18, 55, 59].

Senile systemic amyloidosis (SSA) is associated with elderly patients. This type of disease causes deposits of wild-type transthyretin molecules in myocardium and results in heart failure, atrial arrhythmias and cardiomegaly. The renal symptoms are rare. The prognosis is better than for patients with AL and it is usually few years of survival [19–21].

Diagnostic

The most frequent and the earliest sign in AA type is proteinuria leading to nephrotic syndrome. The only test, which can confirm amyloidosis is biopsy with histological demonstration of amyloid deposits [14]. Congo-red-positive biopsies observed with polarization microscopy are the golden standard in detecting the disease. Amyloid material can be recognised also by gentian violet or thioflavin T [35]. The good method to diagnose a systemic amyloidosis is the subcutaneous adipose tissue biopsy. This is of value in the AL, AA and ATTR, but it has some limitations: unequally distributed deposits, overstaining in Congo red samples with needle biopsies and relatively little involvement of subcutaneous fat tissue in some types of amyloidosis [36]. There are four techniques in typing of amyloid: immunohistochemistry, immunochemistry, mass spectrometry and chemistry. There are 14 proteins detected in samples of amyloidosis, which can be analysed by proteomics. These tests give the investigation of amyloidogenic protein precursors, the identification of the fibrillar deposited proteins, the characterization of the metabolic modifications in affected tissues and the detection of new biomarkers. Amyloid can be classified by antibodies, for example against amyloid P component, AA amyloid, apolipoprotein A-I, lyzosyme, fibrinogen, transthyretin and κ and λ light chains. The immunohistology is most useful for AA and ATTR. The mass spectrometry detects serum monoclonal immunoglobulin free light chains, which are secreted by plasma cells and circulate in plasma and it is useful in AL amyloidosis [37–40].

Treatment

The model of treatment depends on type of amyloidosis. At the 13th International Symposium on Amyloidosis in Groningen updated criteria were given which defined treatment for individual involved organs. The most important is early diagnosis and effective therapy [41].

Treatment of AL is individualized and determined by age, organ dysfunction, present biomarkers and cardiac response. One of the oldest methods is high-dose alkylator-based chemotherapy. It was melphalan with prednisone, later substituted for dexamethasone. Another options are thalidomide, lenalidomide, pomalidomide, bortezomib and colchicine. In some cases autologous stem cell transplantation with earlier chemotherapy has higher response rate than conventional chemiotherapy This method is the standard treatment for multiple myeloma and employed for AL. Medical treatment is also prescribed according to clinical indications and medical judgement and it includes diuresis, serial thoracenteses, pleurodesis, bronchial repermeabilization, avoid digitalis and calcium channel blockers, assessment for atrial thrombi, mechanical pacing [41–44].

AA is associated with other diseases with inflammation and the basis is a common_control of both diseases and decrease of the inflammation. The popular treatment is immunosuppressants: methotrexate, cyclophosphamide and chlorambucil. In some diseases, for example familial Mediterranean fever, the most effective drug is colchicine. The best prognostic factor of therapy is normal renal function. Another effective treatment is demethyl sulfoxide, especially in bowel's inflammatory diseases. There are tested new methods of immunotherapy, which may eliminate deposits from systemic and local amyloidosis [45–50].

In ATTR type the best option is early liver transplantation. The surgery can not prevent all symptoms, cardiomyopathy and neuropathy progress even after transplantation. In cases with advanced organ's damage there is possibility of hepatorenal or hepatocardiac transplantation [51, 52].

For patients with DRA the most important step is renal transplantation, which reduces β_2-microglobulin to the normal level in plasma. The surgery does not decrease radiologic and histologic lesions long time after transplantation. β_2-microglobulin can be removed by hemodialysis with high-flux membranes and

β_2-microglobulin adsorption columns. The established DRA can be treated with low-dose steroids and non steroidal anti-inflammatory drugs [53–55].

Treatment of amyloid cardiomyopathy is primarily the therapy of heart failure. It can involve pharmacotherapy, for example with diuretics, beta blockers, angiotensin enzyme inhibitors, angiotensin receptor blockers or digoxin. The next step is heart transplantation or ventricular assist devices. Anticoagulation decrease risk of intracardiac thrombus, which is associated with amyloid cardiomyopathy [56–58].

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Correspondence address:
Agnieszka Gaczkowska, MD
Poznan University of Medical Sciences
Department of Biochemistry and Molecular Biology
6 Swiecickiego St, 60-781 Poznan, Poland
phone: +48618546511
fax: +48618546510
email: gaczka@gmail.com

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Review articles: These articles should describe recent advances in areas within the Journal's scope. Review articles cannot exceed 5000 words length (excluding title page, abstract and references) and contain no more than a combination of 10 tables and/or figures. Authors are encouraged to restrict figures and tables to essential data that cannot be described in the text. The number of references should not exceed 80.

A THOUSAND WORDS ABOUT... is a form of Mini-Reviews. Manuscripts in this category should focus on *latest achievements of life science and medicine.* Manuscripts should be up to 1000 words in length (excluding title page, abstract and references) and contain up to 5 tables and/or figures and up to 25 most relevant references. The number of authors is limited to no more than 3.

OTHER SUBMISSIONS

Invited Editorials: Editorials are authoritative commentaries on topics of current interest or that relate to articles published in the same issue. Manuscripts should be up to 1500 words in length. The number of references should not exceed 10. The number of authors is limited to no more than 2.

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The Rationale, Design and Methods of New Studies: Manuscripts in this category should provide information regarding the grants awarded by different founding agencies, e.g. National Health Institute, European Union, National Science Center or National Center for Research and Development. The manuscript should be presented in the format of Research Project Objectives, Research Plan and Basic Concept, Research Methodology, Measurable Effects and Expected Results. The article should also contain general information about the grant: grant title, keywords (up to five), name of the principal investigator and co-investigators, founding source with the grant number, *Ethical Committee permission number,* code in clinical trials (if applicable). Only grant projects in the amount over 100,000 Euro can be presented. Manuscripts should be up to 2000 words in length (excluding references) and can include up to 5 tables and/or figures. The abstract should not exceed 150 words. The number of authors is limited to the Principal Investigator and Co-investigators.

Acknowledgements

Under acknowledgements please specify contributors to the article other than the authors accredited. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.). Also acknowledge all sources of support (grants from government agencies, private foundations, etc.). The names of funding organizations should be written in full.

References

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

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

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

Some examples

Standard journal articles

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

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

Books

Personal author(s)

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

Editor(s) or compiler(s) as authors

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

Chapter in the book

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

TABLES: Tables should be typed on sheets separate from the text (each table on a separate sheet). They should be numbered consecutively with Arabic numerals. Tables should always be cited in text (e.g. table 2) in consecutive numerical order. Each table should include a compulsory, concise explanatory title and an explanatory legend. Footnotes to tables should be typed below the table body and referred to by superscript lowercase letters. No vertical rules should be used. Tables should not duplicate results presented elsewhere in the manuscript (e.g. in figures).

FIGURES: All illustrations, graphs, drawings, or photographs are referred to as figures and must be uploaded as separate files when submitting a manuscript. Figures should be numbered in sequence with Arabic numerals. They should always be cited in text (e.g. figure 3) in consecutive numerical order. Figures for publication must only be submitted in high-resolution TIFF or EPS format (*minimum 300 dpi resolution).* Each figure should be self-explanatory without reference to the text and have a concise but descriptive legend. All symbols and abbreviations used in the figure must be defined, unless they are common abbreviations or have already been defined in the text. Figure Legends must be included after the reference section of the Main Text.

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SUPPLEMENTARY ONLINE MATERIAL: Authors may submit supplementary material for their articles to be posted in the electronic version of the journal. To be accepted for posting, supplementary materials must be essential to the scientific integrity and excellence of the paper. The supplementary material is subject to the same editorial standards and peer-review procedures as the print publication.

Review Process

All manuscripts are reviewed by the Editor-in-Chief or one of the members of the Editorial Board, who may decide to reject the paper or send it for external peer review. Manuscripts accepted for peer review will be blind reviewed by at least two experts in the field. After peer review, the Editor-in-Chief will study the paper together with reviewer comments to make one of the following decisions: accept, accept pending minor revision, accept pending major revision, or reject. Authors will receive comments on the manuscript regardless of the decision. In the event that a manuscript is accepted pending revision, the author will be responsible for completing the revision within 60 days.

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