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Original Scientific Article

METABOLIC PARAMETERS IN TYPE 2 DIABETIC PATIENTS WITH POSITIVE CANDIDA CULTURES

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Abstract. *The gut microbiota plays an important role in host metabolism, immunity, digestibility and even behaviour. Candida spec. is common resident of the gastrointestinal tract and integral part of the microbiota. The aim of the study was to evaluate if positive Candida cultures in the stool influence metabolic parameters in type 2DM patients. 46 patients with type 2 DM and oral antidiabetic treatment, were divided into a study group (S=18 patients with positive Candida sp. cultures in stool) and the control group (C= remaining 28 patients). Besides medical history and clinical examination, all patients were tested for coproculture, fasting glycaemia (FPG), HbA1C, total cholesterol (CL) triglycerides (TG), high- density lipoproteins (HDL) and low- density lipoproteins (LDL). Study group patients had a significantly higher BMI (31.41 ± 5.29 vs. 25.18 ± 3.58; p<0.001); HbA1C (9.8 % ± 1.74 vs 6.9% ± 1.89; p<0.05) as well as FPG (10.87 ± 1.35 vs 7.47 ± 1.03; p<0.01), compared to the control group. Even though the study group patients had higher TG, CL, LDL and HDL, compared to the control group, there was no statistical significance verified. Uncontrolled glucoregulation is one of the host condition which favours candida colonization and subsequent infection. This may be related to the decrease in commensal bacteria, probably as the result of yeast-bacterial competition. On the other hand, we have to keep in mindthat a significantly increased number of Candida colonies can affect the rate of digestion and absorption of carbohydrates and consequently increase the level of glycaemia in patients with diabetes*.

Key words: *Candida, microbiota, diabetes, metabolic disorder, hyperglycaemia*

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycaemia, caused by reduced insulin secretion, reduced biological action of insulin, or both [1]. It is estimated that over 500 million people worldwide suffer from diabetes, among whom approximately 90%, has type 2 diabetes mellitus T2DM [2]. Epidemic proportion of diabetes, progressive course of the disease and link to the numerous micro- and macrovascular complications, are burden for health, economics and society around the world. That is why it is of great importance to determine all factors influencing the occurrence of DM as well as modifying its clinical course. One of such potential factors is bacterial and fungal microbiota of the gastrointestinal tract. Numerous studies, in the last decade, have revealed that the gut microbiota plays an important role in the host metabolism, immunity, digestibility and even behaviour [3]. It is pointed out that bacterial and fungal intestinal microbiota play a potential role in the development and progression of diabetes mellitus [4]. Since the processes of digestion and absorption can modify the occurrence of autoagression, one of the apostrophized causes of DM, gut microbiota influence on the speed of decomposition of complex food components, may potentially influence pathogenesis of DM [5]. Altered microbiota composition can change the speed of digestion and absorption of carbohydrates and substantially lead to the postprandial hyperglycaemia changing the clinical course of DM [6]. Experimental studies on the animal models, demonstrated that gut microbiota may

Candida spec. are considered normal inhabitant of the gastrointestinal tract and integral part of the microbiota. These opportunistic microorganisms commonly colonize cutaneous and mucosal surfaces. The gastrointestinal tract represents the ultimate human reservoir for most *Candida* species [8]. Number of 10 to 10^3 fungal colony forming units (CFU)/g faeces are considered as normal range. However, when mucosal surface is damaged or host immunity is compromised, common commensal *Candida sp.* can be the cause of life-threatening invasive infection. Patients with DM have higher predisposition to fungal infections, including *Candida sp.*, due to several mechanisms, such as: higher salivary glucose levels [9], reduced salivary flow [10], microvascular degeneration, but mostly due to diabetes mellitus immunosuppressive effect.

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Even though, *Candida sp.* are one of the most prevalent fungus in gut microbiota, literature data about frequency in DM patients and interplay between host and *Candida* are limited.

Objectives

This retrospective study is conducted with the following aims:

1. To estimate the frequency of *Candida sp*. in patients with type 2 diabetes mellitus.
2. To evaluate if *Candida sp.* presence influences metabolic parameters in type 2DM patients.

Patients and Methods

The study included 46 patients with type 2 DM, 20 women and 26 men. Based on the positive or negative *Candida sp.* in stool, patients were divided into following groups: study group (+C = 18 patients with positive *Candida* sp. cultures in stool; 39.13%) and control group (-C = remaining 28 patients; 60.87%). The number of CFU/g faeces above 10^3 was considered as positive. Patient's characteristics, age, gender, diabetes duration and therapy (antidiabetic and hypolypemic) are provided in Table 1. All the subjects were white Caucasians. The antidiabetic therapy consisted of oral hypoglycaemic agents, metformin and sulfonylurea (SU) preparations (glimepiride or gliclazide). Besides medical history and clinical examination (including BMI) stool and blood samples were collected from all patients participating in the study. Stool samples were used for *Candida sp.* testing and blood samples for determining the following parameters: fasting plasma glucose levels (FPG), glycated haemoglobin (HbA1c) and lipid profile (total cholesterol (CL), triglycerides (TG), high density lipoproteins (HDL-C) and low density lipoproteins (LDL-C)).

Table 1 Characteristics of the studied patient groups

	Candida positive group (+C) (n = 18)	Candida negative group (-C) (n = 28)
Male : Female (n)	10:8	14:14
Age (yrs.)	68.21 ± 10.4	66.97 ± 9.7
Duration of diabetes (yrs.)	9.89 ± 5.04	10.13 ± 6.21
Diabetes treatment	Metformin + SU (18)	Metformin + SU (28)
Statin therapy*	4 (33.33%)	20 (58.82%)

The presented data are means ± SD.
*significant differences within the two compared groups of patients; ($p < 0.01$) – Mantel- Haenzel Chi-squared test

The main exclusion criteria were antibiotic therapy, administration of probiotics and corticosteroids 30 days before stool sampling.

Statistical data processing and determination of significance between the study and control group were performed in the SPSS program.

Results

The average age of all patients was 67.83 ± 9.72 years. The youngest patient was 30 years old, and the oldest was 77. No statistical significance was found relating to the average age in the study (+C) and control group (-C) (68.21 ± 10.4 vs. 66.97 ± 9.7 years).

In the +C group, most patients (12; 66.67%) had some lipid status disorder (mixed hyperlipidaemia, isolated hypercholesterolemia, or isolated hypertriglyceridemia). The majority of patients had mixed hyperlipidaemia (6; 33.33%), followed by isolated hypercholesterolemia (4; 22.22%), and only 2 patients had isolated hypertriglyceridemia (2; 11.11%).

In the -C group, the number of patients with a lipid status disorder was similar (18; 64.28%). Mixed hyperlipidaemia was present in 28.57% (8 patients), isolated hypercholesterolemia in 21.42% (6 patients), and isolated hypertriglyceridemia in 14.29% (4 patients). The distribution of hyperlipidaemia types is shown in Table 2.

Table 2 Hyperlipidaemia types distribution in the studied patients groups

Lipide profil type (n; %)	Candida positive group (+C) (n = 18)	Candida negative group (-C) (n = 28)
Mixed hyperlipidaemia	6; 33.33%	8; 28.57%
Isolated hypercholesterolemia	4; 22.22%	6; 21.42%
Isolated hypertriglyceridemia	2; 11.11%	4; 14.29%
Normal lipid profile	6; 33.33%	10; 35.71%

Higher values of TG (2.03 ± 1.13 vs. 1.80 ± 0.58), CL (5.23 ± 1.23 vs. 4.73 ± 0.92), LDL-C (3.27 ± 0.75 vs. 2.86 ± 0.83) and HDL-C (1.63 ± 0.25 vs. 1.08 ± 0.44) were verified in +C compared to the -C, but without statistical significance (Table 3).

Table 3 Total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol in the studied patients groups.

Parameter	Candida positive group (+C) (n = 18)	Candida negative group (-C) (n = 28)
CL (mmol/l)	5.23 ± 1.23	4.73 ± 0.92
TG (mmol/l)	2.03 ±1.13	1.80 ± 0.58
HDL-C (mmol/l)	1.63 ± 0.25	1.08 ± 0.44)
LDL-C (mmol/l)	3.27 ± 0.75	2.86 ± 0.83

A significantly smaller number of patients in the +C group (4) had statin therapy, compared to the -C group (20) of patients (33.33% vs. 58.82%, $p<0.01$) with high correlation degree between statins and a negative *Candida sp.* findings.

Study group patients had a higher BMI (31.41 ± 5.29 vs. 25.18 ± 3.58; $p<0.001$), HbA1C (9.8 % ± 1.74 vs 6.9% ± 1.89; $p<0.05$) as well as FPG (10.87 ± 1.35 vs. 7.47 ± 1.03; $p<0.01$) compared to the control group (Table 4).

Table 4 Laboratory and antropometric parameters (HbA1C, FPG and BMI) in studied patients

Parameter	Candida positive group (+C) (n = 18)	Candida negative group (-C) (n = 28)
HbA1C[#] (%)	8.02 ± 1.74	7.9 ± 1.89
FPG[§] (mmol/l)	7.87 ± 1.35	7.47 ± 1.03
BMI[*] (kg/m2)	31.41 ± 5.29	25.18 ± 3.58

The presented data are means ± SD.

[#]significant differences within the two compared groups of patients; ($p < 0.05$)

[§]significant differences within the two compared groups of patients; ($p < 0.01$)

*significant differences within the two compared groups of patients; ($p < 0.001$)

Discussion

Human microbiota represents the complete set of microorganisms in the human body, while the term microbiome defines total genetic material of the microbiota. Such a powerful "microcosm" carries a huge genetic information, which is 150 times larger than the human genome. Precisely for this reason, the concept of microorganisms being either pathogenic or commensal is slowly being abandoned. An increasing number of studies confirm that it is a matter of complex interactions between microbiota and humans, which have a very significant impact on metabolism, the immune system, and even on the host's behaviour [3]. The knowledge that microbiota can modulate hormonal secretion was the foundation for a new field known as microbiological endocrinology.

Candida belongs to a group of opportunistic microorganisms that are normal inhabitants of the skin and mucous membranes. In faeces, the number of *Candida sp.* colonies should not exceed $10\text{-}10^3$. However, when the anatomical barrier is violated, candidiasis occurs, which can significantly threaten the life of the host [11]. *Candida albicans* infection is the most widespread invasive mycosis with a mortality rate of up to 40% [12, 13].

Our study results showed that 26% of included patients had positive *Candida sp.* in the stool, which is a slightly higher percentage, compared to the data from the literature [11]. Dietary habits in the region from which the study patients are, consist of many products important for *Candida sp.* growth. Food such as bread (containing yeast), white flours, smoked or cured pork meat (containing mould), wine and beer (alcohol which is fermented using yeast), sugar itself and dried fruits (which have been jarred) are well-known to make a suitable environment for *Candida sp.* overgrowth. It has been described that *Candida sp.* is more frequent in the faeces of patients with type 1 and type 2 DM with poor glycemic control as opposed to healthy subjects [4]. In our study, all patients were type 2 DM and significantly higher HbA1C and FPG in the *Candida positive* group pointed out that poor glycaemic control favours development of the candidiasis among DM patients. There are several factors largely influencing the balance between host and yeasts, favoring the transition of *Candida* sp. from commensal to pathogen and causing infection. One of the main reasons is direct effect of elevated blood glucose levels, creating specific conditions for intensive fungal colonization [4]. *Candida sp.* uses glucose as a source of energy, necessary to synthetize biofilms and the polysaccharide matrix as protection from the environment [14, 15]. Our study results also showed that Candida positive group of patients had significantly higher BMI. The compositional changes of intestinal fungi have been found in patients with obesity [16]. Also, different methods confirmed correlation between increased *Candida sp.* and obesity [17]. However, causal relationship between intestinal fungi and obesity has only been confirmed in the animal experimental model. *Sun et al.* in their experimental study showed that the treatment with amphotericin B and fluconazole significantly repressed the progress of obesity in mice [18]. Further investigations in this field will certenly give the answers about physiological functions and underlined mechanism of intestinal fungi during the development of obesity.

The patients of the Candida positive group, had higher levels of CL, TG, LDL-C and HDL-C compared to the Candida negative control group, but without statistical significance. There are opposite literature data regarding the correlation between *Candida* and lipid status. Gosiewski et al. in their research, verified a negative correlation between *Candida* in the large intestines and serum lipid levels in T2DM subjects, while Netea et al. in the experimental study, pointed out that hyperlipoproteinaemia enhances susceptibility to acute disseminated *Candida albicans* infection [4, 19]. We have to emphasize that in our study patients, significantly higher number of patients in Candida negative group were using statin therapy (58.82% vs. 33.33%). There is a growing evidence that statin therapy has ability to reduce the incidence of positive culture of *Candida spec.* By inhibiting 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, statins lead to the reduction of mevalonate, thereby reducing cholesterol levels in humans and possibly ergosterol in fungi. Considering that ergosterol is an essential lipid component of the cell membrane of fungi, they will be more vulnerable especially to the action of antifungal drugs [11, 20]. Although our study included a relatively small number of patients, the results are consistent with the literature data, showing that statin therapy in patients with diabetes is important not only in reducing the concentration of CL and TG, but also in providing some protection against the intestinal *Candida* overgrowth.

The classic, well-known role of intestinal microbiota is in the digestion of carbohydrates and their fermentation to fatty acids. Hyperglycaemia and poor glycaemic control in DM, favour *Candida sp.* overgrowth. On the other hand, increased number of *Candida* colonies can affect the speed of digestion and absorption of carbohydrates leading to the higher postprandial glycaemia levels in DM patients [6]. Microbial endocrinology, reveals that the intestinal microbiota affects neuropeptides such as

ghrelin, insulin and leptin, crucial controllers of appetite and metabolism. Altered microbiota could be one of the underlying pathophysiological mechanism of obesity [7, 21].

Our study limitations are the sample size of the study groups, inclusion individuals with relatively long DM duration and single-point examination. Thus, the obtained results require confirmation on a greater number of patients.

Conclusion

Candida fungi appear to be more prevalent in the T2DM patients with poor glycemic control, higher FPG and higher BMI. Statin therapy is strongly correlated with negative *Candida* findings. Although microbiological endocrinology is a relatively new field, there is growing evidence of the intestinal microbiota importance and its influence on metabolism, appetite, immunity, reproduction and behaviour. Further research should elucidate these complex interactions between the host's endocrine system and gut flora. The development of preventive and therapeutic measures such as probiotics, statins and certain dietary products would certainly be of great help in better understanding of diabetes.

List of abrreviations

DM - Diabetes mellitus
T2DM - type 2 diabetes mellitus
T1DM - type 1 diabetes mellitus
BMI - Body mass index
FPG - fasting plasma glucose
HbA1c - glycated haemoglobin
CL - total cholesterol
TG - triglycerides
HDL-C - high density lipoprotein cholesterol
LDL-C - low density lipoprotein cholesterol
CFU - colony forming units

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Original Scientific Article

ASSESSMENT OF THE INSTRUMENTAL ACTIVITIES OF DAILY LIVING IN MILD COGNITIVE IMPAIRMENT AND DEMENTIA DUE TO ALZHEIMER'S DISEASE: DIAGNOSTIC ACCURACY OF THE SERBIAN VERSION OF THE AMSTERDAM IADL QUESTIONNAIRE

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Abstract. *To establish a diagnosis of dementia, it is necessary, in addition to cognitive impairment, to prove the existence of a disorder of instrumental activities of daily living (IADL). The Amsterdam IADL Questionnaire is a reliable instrument translated into different languages. This study aims to assess the diagnostic accuracy of the Serbian version of the Amsterdam IADL Questionnaire. The study included 75 patients with mild cognitive impairment (MCI) and dementia due to Alzheimer's disease. The questionnaire was scored using the weighted average (WA) and item response theory (IRT) scoring method. Diagnostic accuracy was examined using receiver–operating characteristic (ROC) curves. The area under the curves (AUC) was calculated with 95% confidence intervals (CI). The correlation between IRT and WA scores was strong and significant ($r=-0.980$, $p<0.001$). The AUC for the IRT scores of A-IADL-Q was 0.832 (95% CI: 0.729 to 0.909), while the AUC for the WA scores of A-IADL-Q was 0.848 (95% CI: 0.746 to 0.920). Both were significantly different from the AUC of 0.5 ($p<0.001$). There was no significant difference between the AUCs of IRT and WA scoring ($z=1.157$; $p=0.247$). Cutoffs and the highest combination of sensitivity and specificity for the IRT (sensitivity 0.767; specificity 0. 844) and WA (sensitivity 0.744; specificity 0. 844) scores of A-IADL-Q were calculated. We have shown that A-IADL-Q has moderate diagnostic accuracy in differentiating dementia and MCI. This instrument can be used in combination with cognitive measures to diagnose dementia in its early stages*.

Key words: *Alzheimer's disease, Dementia, instrumental activities of daily living, Mild cognitive impairment (MCI)*

Introduction

In order to establish a diagnosis of dementia, it is necessary, in addition to cognitive impairment, to prove the existence of a disorder of instrumental activities of daily living (IADL) [1,2]. IADLs are complex everyday activities (like managing finances, using devices, public transportation, etc.) that become disrupted in early dementia [3]. Different questionnaires have been used to detect impairment of IADL [4]. However, the Amsterdam IADL questionnaire® (A-IADL-Q) was developed with an aim to have appropriate psychometric characteristics in both its standard form (with 70 questions) [3,5] and short form (30 questions) [6]. A-IADL-Q has been translated into and adapted for 31 languages, including Serbian [7]. The Serbian version of the short form of A-IADL-Q is a reliable and valid measure of IADL in patients with dementia and mild cognitive impairment, as shown in our previous study [8]. A-IADL-Q was administered using the Qualtrics online platform. Informers (friends and family members of patients) answer questions about 30 different everyday activities.

This instrument has different scoring methods, including weighted average (WA) and item response theory (IRT) scoring. The WA scoring is a simpler method, where total scores are obtained directly from the Qualtrics online platform. The IRT scores have better distribution and absence of floor and ceiling effect. However, the IRT scores have to be calculated separately in a process that delays clinical diagnosis [6].

This study is an extension of our previously published research [8], where we performed a reliability analysis (by assessing internal consistency and reproducibility) and evaluated the construction validity of the Serbian version of A-IADL-Q. The present study aims to assess the diagnostic accuracy of the Serbian version of the Amsterdam IADL Questionnaire as a screening tool for dementia and to determine the cutoffs with the best combination of sensitivity and specificity.

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Materials and Methods

The study included 75 patients with cognitive impairment: 32 patients with MCI and 43 with dementia due to AD. All patients were recruited at the Outpatients Department of the Clinic of Neurology, University Clinical Center Niš.

The diagnoses of probable dementia due to AD and MCI were established using the appropriate National Institute of Aging and Alzheimer's Association (NIA-AA) criteria [1,9]. A neurologist examined all patients, performed cognitive screening (mini-mental state examination (MMSE) [10]), and referred them for further laboratory, neuroimaging, and neuropsychological testing. Patients with severe dementia (MMSE <10) and those with motor impairment were excluded from the study.

This study protocol was reviewed and approved by the Ethical Board of the University Clinical Center Niš, and the Ethical Committee of the Medical faculty University of Niš. All patients and/or caregivers provided written informed consent. All the work with human subjects has been conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

A-IADL-Q [6,8] was completed by informers (close friends or relatives) of patients using the Qualtrics online platform (www.qualtrics.com). The A-IADL-Q short form consists of 30 items, and each item has a 5-point scale response option. The questionnaire was scored using the weighted average (WA) and item response theory (IRT) scoring methods.

The WA scoring method is implemented in the online platform, where it is calculated by dividing the total IADL score by the number of items assessed (value between 0 and 4) and multiplying it by 25. The WA A-IADL-Q score ranges from 0 to 100, where a higher score represents a more pronounced IADL impairment.

Scoring based on Item Response Theory (IRT) takes into account the different "difficulty" of items of the A-IADL-Q. Impairment of more complex activities contributes more to the total score than impairment of simpler activities [11]. The total score has a normal distribution with a mean of 50, a standard deviation (SD) of 10, and ranges from about 20 to 80, with lower scores representing poorer performance [11]. IRT scoring is described in more detail elsewhere [6].

WA and IRT A-IADL-Q scores, years of education, and age were compared in patients with dementia and those with MCI using the independent t-test. Gender distribution was explored using the chi-squared test. The correlation between different scoring methods was analyzed by the Pearson correlation coefficient.

Diagnostic accuracy was examined using receiver–operating characteristic (ROC) curves created for both the WA and IRT A-IADL-Q scores. The minimal sample size for the area under the ROC curve analysis was estimated with a power of 80% and a significance level of 5% [12]. We have calculated the area under the curves (AUC) with 95% confidence intervals (CI). The AUCs were classified as having low (0.50 - 0.70), moderate (0.71 - 0.90), and high accuracy (>0.90) [13]. The best possible cutoff score with the highest combination of sensitivity and specificity was determined using the Youden index, calculated by deducting 1.0 from the sum of sensitivity and specificity [14]. AUCs of different ROC curves were compared using the DeLong method [15].

Results

Demographic parameters are presented in Table 1. There was no statistically significant difference in gender distribution between the two diagnostic groups ($\chi^2 = 0.12$, p=0.914). MCI group had a higher level of education (χ^2=7.137; p=0.028) and longer average education in years than the dementia group (t=3.208; p=0.002). The dementia group was significantly older than the MCI group (t=-2.615; p=0.011).

Table 1 Demographic parameters and Mini-mental state examination (MMSE) score of the patients with AD dementia and mild cognitive impairment (MCI)

	AD Dementia (N=43)	MCI (N=32)	Total (N=75)
Gender N (%)			
Female	25 (58.1%)	19 (59.4 %)	44 (58.7%)
Male	18 (41.9%)	13 (40.6%)	31 (41.3%)
Age (years) (M±SD)	76.58±7.02	70.81±11.98	74.12±9.81
MMSE	14.81±3.11	22.34±2.36	18.03±4.68
Education			
Primary level	24 (55.8%)	8 (25.0%)	32 (42.7%)
Secondary level	9 (20.9%)	11 (34.4%)	20 (26.7%)
Tertiary level	10 (23.3%)	13 (40.6%)	23 (30.6%)
Years of education (M±SD)	9.14±4.91	12.53±3.95	10.59±4.80

There was a statistically significant difference in MMSE scores between the two groups (t=11.445; p<0.001). A statistically significant difference was registered regarding both the WA (t=-6.059; p<0.001) and the IRT A-IADL-Q scores (t=5.670; p<0.001).

The AUC for the IRT scores of A-IADL-Q was 0.832 (95% CI: 0.729 to 0.909), which is significantly different from the AUC of 0.5 (z=7.236; p<0.001). The calculated AUC for the IRT scores showed moderate accuracy according to the Swets criteria.

The AUC for the WA scores of A-IADL-Q was 0.848 (95% CI: 0.746 to 0.920). It was significantly different from the AUC of 0.5 (z=7.948; p<0.001), reflecting moderate accuracy according to Swets criteria.

The difference between AUCs of IRT and WA scoring was 0.015 (95% CI: -0.011 to 0.041), which is not statistically significant (z=1.157; p=0.247) according to the DeLong method (shown in Fig. 1).

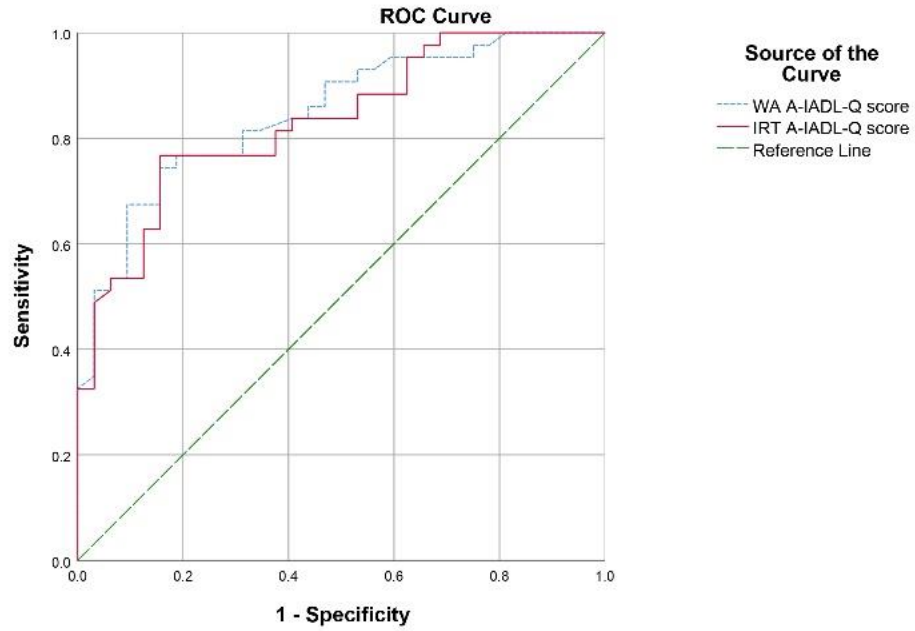

Fig. 1 Receiver–operating characteristic (ROC) curves created for the weighted average (WA) and item response theory (IRT) Amsterdam-IADL-Questionnaire (A-IADL-Q) scores

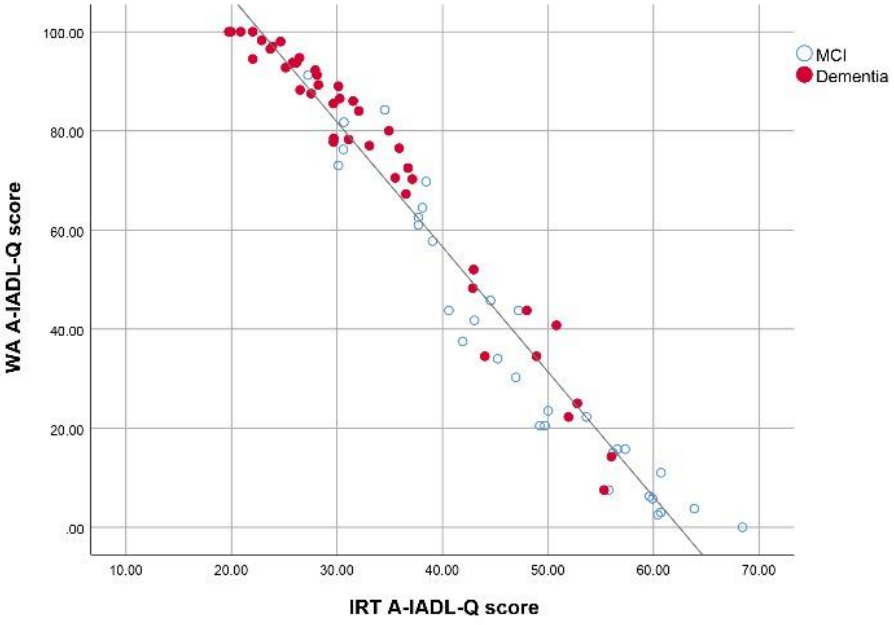

Fig. 2 Correlation (scatter plot) of the weighted average (WA) and item response theory (IRT) Amsterdam-IADL-Questionnaire (A-IADL-Q) scores in patients with dementia and mild cognitive impairment (MCI)

We have calculated the sensitivity and specificity values for the IRT and WA scores of the A-IADL-Q for different cutoff scores. The WA cutoff score of 69.75 points (Younden index = 0.588) had the highest combination of sensitivity 0.744 (95% CI: 0.588 – 0.865) and specificity 0. 844 (95% CI: 0.672 – 0.947). For the IRT scores, the highest combination of sensitivity (0.767 (95% CI: 0.614 – 0.882)) and specificity (0.844 (95% CI: 0.672 – 0.947)) was registered at the cutoff score of 37.146 (Younden index = 0.611).

The correlation between IRT and WA scores was strong and highly significant (r=-0.980, p<0.001) (shown in Fig. 2).

Discussion

Our study explored the diagnostic accuracy of two different scoring methods of A-IADL-Q in discriminating between dementia and MCI. Using both scoring methods, we have shown moderate diagnostic accuracy. It should be kept in mind that this questionnaire was not designed as an independent diagnostic tool for diagnosing dementia. However, in earlier studies, a diagnostic utility of the original version of A-IADL-Q was explored, and the cutoff score of 51.4 (using the IRT scoring method) with the best combination of sensitivity and specificity was calculated [16].

Our study showed a significantly lower cutoff A-IADL-Q IRT score than previous research (37.8 vs. 51.4) [16]. This difference could be a consequence of cultural influences, different characteristics of the sample in these two studies, and differences in the diagnostic approach when establishing a diagnosis of dementia.

Cultural differences may influence the level of IADL performance that is considered abnormal. Expectations regarding the activities of older adults within different societies can vary and lead to a lower probability of the recognition of dementia by the patient's family members and community in lower and middle-income countries such as Serbia [17]. Also, living in the urban centers of a developed country might objectively require more complex skills in everyday life than living in a smaller town or rural environment of a developing country [18].

Previous research has shown that a level of education in the general population is significantly correlated with knowledge about dementia [19] and that a lower level of knowledge about dementia can hamper timely access to medical care [20]. As the significant difference in the level of education between the two groups of patients was registered in our sample, there may be a selection bias where patients with a higher education turn to a neurologist and accept cognitive screening for less pronounced problems with cognition than patients with lower education [20,21]. The dementia group in our sample was less educated and had a lower MMSE score and a greater IADL impairment than the corresponding group in the previous research by Sikkes and colleagues [16].

Finally, a different approach to measuring cognitive impairment might explain the differences in cutoff scores. MMSE, the primary screening instrument for patients with cognitive complaints in our sample, has its limitations. Lower sensitivity leads to diagnosing dementia later than in the cases where more sensitive neuropsychological methods were used to assess cognitive impairment [22].

The diagnosis of dementia requires the clinician to differentiate this diagnostic entity from mild cognitive impairment [1]. As patients with normal cognition and severe dementia were excluded from our sample, we examined the diagnostic accuracy under conditions corresponding more closely to actual clinical practice. This approach may also explain the difference in our results compared to previous research.

A recent study in the Netherlands proposed an IADL impairment categorization related to the total IRT score of the A-IADL-Q as follows: normal (scores ≥60), mild (scores 50–59), moderate (scores 40–49) and severe (scores < 40) [11]. The authors of the proposed classification note that it represents the consensus of caregivers and clinicians in their study and that the interpretation of cut-offs may depend on individual definitions and opinions [11]. However, according to the suggested values, the cut-offs determined in our study belong to the severe IADL disorder. Considering that the A-IADL-Q was not used as a diagnostic criterion in our study, our cutoff value indicates that examining IADL based on the clinician's opinion only significantly underestimates the deficit. Objectively measured IADL using the appropriate instrument could help to diagnose dementia in an earlier phase.

ROC curve analysis didn't show a significant difference between the two methods of A-IADL-Q scoring. Both scoring methods showed similar sensitivity and specificity. The advantages of the IRT scoring method are better psychometric characteristics and a less strong ceiling effect [6]. However, WA scoring is much simpler, and this score is already available in the online platform used for IADL assessments. The authors of the validation study of the UK version of A-IADL-Q also used the WA scoring method in part of their study, which was in high concordance with the IRT scoring [23]. Our study showed that both scoring methods could be used in clinical practice and research with similar sensitivity and specificity.

The limitation of this study is the use of MMSE as a sole measure of cognition in the majority of patients, as well as the relatively small sample of patients. Also, we have included just one dementia type, dementia due to AD. Analysis of this instrument's characteristics in dementia syndromes other than Alzheimer's disease could be the aim of further studies.

Conclusion

In conclusion, we have shown that A-IADL-Q has moderate diagnostic accuracy in differentiating dementia and mild cognitive impairment. This instrument can be used in combination with cognitive measures to diagnose dementia in its early stages.

Acknowledgments: The Amsterdam IADL Questionnaire ©, 2010, VU University Medical Center, Alzheimer Center Amsterdam; All rights reserved. The Amsterdam IADL Questionnaire can be obtained from the developers after registration and is free for use in all academic, not-for-profit research and public health agencies.
(https://www.alzheimercentrum.nl/professionals/amsterdam-iadl/).
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Original Scientific Article

SECONDARY HOST PLANTS OF WATER LILY APHID, *RHOPALOSIPHUM NYMPHAEAE* (HEMIPTERA: APHIDIDAE) IN SERBIA

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Abstract. *The water lily aphid is a cosmopolitan species that feeds on various plants. Its primary hosts are Prunus species from which they migrate to their secondary hosts – aquatic and semi-aquatic plants. So far, in Serbia, Rhopalosiphum nymphaeae is recorded to attack only three plant species as secondary hosts. In our study, conducted in September 2020, we have researched the association of water lily aphid with secondary hosts in wetland habitats. A total of 44 samples were collected from 16 localities. In addition to the plant species previously reported, in this study 11 secondary hosts are documented for the first time in Serbia. The most common trophic association of R. nymphaeae was with Salvinia natans which was registered in 13 localities. There is a high possibility of finding new records of secondary hosts, therefore, more research is needed to complete the information about the water lily aphid and its hosts in Serbia.*

Key words: *trophic associations, wetlands, aquatic and semi-aquatic host plants*

Introduction

The water lily aphid, *Rhopalosiphum nymphaeae* (L.) (Hemiptera: Aphididae) is a polyphagous heteroecious aphid, with a holocyclic life cycle and cosmopolitan distribution. This species can produce different morphs in temperate conditions: parthenogenetic apterae, alatae, and sexual forms [1]. Its primary hosts are usually various *Prunus* species, where it feeds on young twigs, leaf petioles, and fruit stalks. *Rhopalosiphum nymphaeae* can cause leaf curling and deformation, and it can slow down the growth and dry the plants [1]. In spring, they migrate to secondary hosts such as *Alisma* L., *Callitriche* L., *Juncus* L., *Lemna* L., *Nuphar* Sibth. & Sm., *Nymphaea* L, *Potamogeton* L., *Spirodela* Schleid., *Typha* L., etc. [1]. Up until late summer, parthenogenetic apterae and alatae develop on secondary hosts for which they always choose aquatic and semi-aquatic plants [2,3]. Because of overcrowding, competition, and low feeding resources, sexual forms migrate back to their overwinter hosts where they lay eggs [3]. Apterae are brown on the primary host and reddish-brown turning dark olive on the secondary one [1].

Apart from living on aerial plant organs of its secondary hosts, *R. nymphaeae* can also survive in underwater conditions [3]. It has specialized body hairs that can trap air, enabling it to feed on submerged plant parts [4]. Using their stylets to probe plant sap they select the most suitable host among them and usually concentrate along the petiole, leaf lamina, buds, and flowers of water lilies and lotus. The leaves then become curled and small, often with deposits of honeydew on the leaf surface, which can cause the development of black sooty mould [3]. After colonizing aquatic plants, water lily aphids reproduce quickly and can be very destructive. *Rhopalosiphum nymphaeae* can give one offspring every six hours [5], and cover almost all plants in the water a few weeks after their first appearance [6].

Rhopalosiphum nymphaeae is widely distributed on a variety of hosts; for example, in Europe, its reported primary hosts are species from the Rosaceae family: *Amygdalus communis* L. [7], *P. armeniaca* Marsh., *P. avium* L., *P. persica* (L.), *P. spinosa* L., while it can have more than 100 different secondary hosts such as *Azolla filiculoides* Lam., *Calla palustris* L., *Lemna minor* L., *Nymphaea odorata* Aiton, *Potamogeton crispus* L., and *Typha angustifolia* L. [8]. During almost 30 years of studies of aphid fauna, and fauna of parasitoid wasps from the subfamily Aphidiinae in Serbia, *R. nymphaeae* was only found sporadically, because it was never a research target species. Petrović-Obradović [9] reported water lily aphid on its primary hosts, *Prunus spinosa* L., *P. domestica* L., *P. cerasifera* Ehrh., and *Prunus* sp., and its two secondary hosts (*Alisma plantago-aquatica* and *Ranunculus aquatilis*). Another research reported that *R. nymphaeae* was most commonly found on *Typha latifolia* [10,11,12]. It was also found on *Alisma* sp. [10].

Many of water lily aphids' secondary hosts are weeds and invasive plants [8]. Its successful usage in biological control has not been reported yet, but some authors (e.g., [13]) recommended it as a biological control agent against duckweed, *Heteranthera limosa* (Sw.) Willd., a pest of rice crops in California. Tomanović et al. [14] assume that water lily aphid can significantly impact the

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control of pest populations in neighboring wetland systems. Here it should be pointed out that water lily aphid is a reservoir of many parasitoid species, primarily Aphidiinae (Hymenoptera: Braconidae) which are known to be effective natural enemies of some important aphid pests [12]. Some of the parasitoids reported on *R. nymphaeae* are *Aphidius colemani* Viereck, *A. matricariae* Haliday, *A. rhopalosiphi* de Stefani-Perez, *Diaeretellus palustris* Starý, *Lysiphlebus fabarum* (Marshall), *Trioxys auctus* (Haliday) and *Praon necans* Mackauer [2,12,15]. It was also found that some predatory insects were reported to be serious natural enemies of the studied aphid, such as various coccinellids (*Brumoides suturalis* Fabricius, *Coccinella septempunctata* L., *Micraspis discolor* Fabricius, *Menochilus sexmaculatus* Fabricius and *Scymnus* Kugelann species) [16], a syrphid *Ischiodon scutellaris* Fabricius [17], common green lacewing *Chrysoperla carnea* (Stephens) [18] and also the very well studied aphid midge *Aphidoletes aphidimyza* Rondani. In the end, some entomopathogenic fungi e.g., *Lecanicillium lecanii* (Zimm.) were found to be natural enemies of the water lily aphid [3].

Since there was no systematic investigation on the spectrum of secondary hosts of *R. nymphaeae* in Serbia, we assumed that the number of secondary hosts would have been higher. The main goal of this research is to provide a list of aquatic plants attacked by water lily aphid and to detect the prevalence of the secondary host choice.

Materials and Methods

The material for this research representing *apterae* and *alatae* (Figure 1) was collected in September 2020, from 16 localities in wetland habitats in northern Serbia near the flow of the Danube River (Figure 2). Parts of plants infected with aphids were cut and put in plastic containers 10x15 cm, covered with muslin cloths, and transported to the laboratory. Eleven different plant species were identified on-site and were not kept for further research. Both apterae and alatae were put in 2 ml plastic tubes filled with 70% ethyl alcohol and labelled. Identification was done using a binocular stereomicroscope (Leica DFC 320, M216A). The key of Blackman & Eastop [1] was used as very reliable for the identification of collected adult apterous vivipaours females. Some data on the secondary host (*Alisma plantago-aquatica*, *Alisma* sp., *Ranunculus aquatilis*, *Typha latifolia*) were extracted from previously published papers [9–12, 19]. Aphid material was deposited at the Faculty of Sciences and Mathematics, Department of Biology and Ecology, University of Niš.

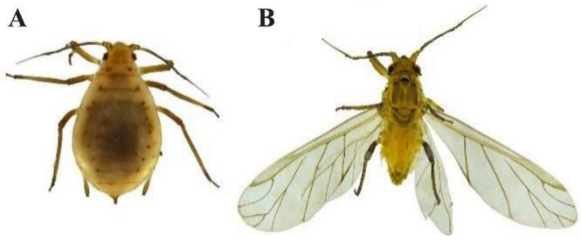

Fig. 1 Apterous (A) and alatae (B) vivipare female of R. nymphaeae – dorsal view. The photos were taken on a Leica M165C camera with 25x magnification.

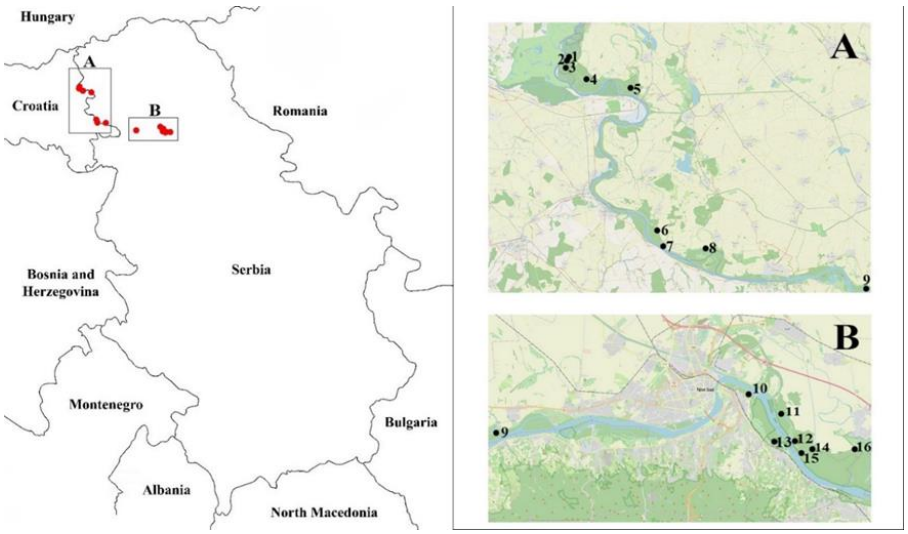

Fig. 2 Maps of the research area. The higher resolution map shows the overlapping localities more closely: **(A)** localities 1-9, **(B)** localities 9-16. The numbers represent the following localities with DMS coordinates: 1. Markova bara (45° 35' 35.88" N, 18° 55' 8.04" E); 2. Osmica (45° 35' 26.16" N, 18° 54' 57.6" E); 3. Tošina bara (45° 34' 39.72" N, 18° 54' 44.64" E); 4. Bara kod tri mosta (45° 33' 30.24" N, 18° 57' 37.44" E); 5. Tuškoš (45° 32' 40.92" N, 19° 3' 43.56" E); 6. Doktor pumpa (45.3131, 19.1232); 7. Hašaški Dunavac (45.2882, 19.1353); 8. Lovrenac (45° 17' 7.44" N, 19° 14' 11.4" E); 9. Begečka jama (45° 13' 16.68" N, 19° 36' 14.76" E); 10. Petrovaradinski Dunavac (45° 15' 9" N, 19° 53' 39.12" E); 11. Patrijaršijska tonja (45° 14' 8.88" N, 19° 55' 52.32" E); 12. Agla (45° 12' 53.28" N, 19° 56' 49.56" E); 13. Karlovački Dunavac (45° 12' 48.96" N, 19° 55' 27.84" E); 14. Bara sa posipom (45° 12' 27" N, 19° 58' 4.08" E); 15. Rupa (45° 12' 17.28" N, 19° 57' 19.44" E); 16. Šlajz (45° 12' 27" N, 20° 0' 57.96" E). The sampling map was made in QGIS 3.22.3.

Results

A total of 44 samples of *Rhopalosiphum nymphaeae* were collected from 11 different aquatic and semi-aquatic plants. The hosts were presented in alphabetical order. The data on the date of sampling are given chronologically. Abbreviations for legators are given in brackets: [DC] Dušanka Cvijanović, [DM] Darija Milenković; abbreviations for aphid forms are given in parentheses: (al) alatae, (ap) apterae, (n) nymphs.

Hydrocharis morsus-ranae **L.,** Hydrocharitaceae**:** 09.09.2020. Novi Sad, Koviljski rit, Agla, (ap 6, n 10) ; 09.09.2020. Novi Sad, Petrovaradin, Karlovački Dunavac (ap 2a, n 6) [DM].

Nuphar lutea Smith, Nymphaeaceae: 09.09.2020. Novi Sad, Koviljski rit, Patrijaršijska tonja (ap 7, n 63); 10.09.2020. Bačko Novo Selo, Doktor pumpa (n 6); 11.09.2020. Apatin, Tošina bara (al 2, ap 2, n 11) [DM].

Nymphaea alba L., Nymphaeaceae: 08.09.2020. Novi Sad, Koviljski rit, Šlajz (ap 9, n 36); 09.09.2020. Novi Sad, Koviljski rit, Agla (ap 2, n 5); Novi Sad Koviljski rit, Patrijaršijska tonja (al 14, ap 15, n 42) [DM].

Nymphoides peltata (S. G. Gmel.) Kuntze, Menyanthaceae: 09.09.2020. Novi sad, Koviljski rit, Agla (ap 16, n 42); 10.09.2020. Bačko Novo Selo, Hašaški Dunavac (al 7, ap 3, n 23); 11.09.2020. Apatin, Bara kod tri mosta (ap 1); Apatin, Osmica; Apatin, Tošina bara (ap 8, n 59); Apatin, Tuškoš (al 1, ap 5, n 21) [DM]; 18.09.2020. Novi Sad, Begeč, Begečka jama (ap 9, n 44) [DC].

Phragmites australis (Cav.) Steud., Poaceae: 11.09.2020. Apatin, Tošina bara (ap 7, n 20) [DM].

Polygonum **sp.,** Polygonaceae**:** 09.09.2020. Novi Sad, Koviljski rit, Agla (ap 2, n 5) [DM].

Persicaria amphibia (L.), Polygonaceae: 09.09.2020. Novi Sad, Koviljski rit, Patrijaršijska tonja (ap 6, n 13), 11.09.2020. Apatin, Tošina bara (ap 2, n 8) [DM].

Salvinia natans (L.), Salviniaceae: 09.09.2020. Novi Sad, Koviljski rit, Agla (ap 1, n 3); Novi Sad, Koviljski rit, Bara sa posipom (al 8, n 2); Novi sad, Koviljski rit, Patrijaršijska tonja (al 1, ap 7, n 12); Novi Sad, Petrovaradin, Karlovački Dunavac (ap 2, n 14); Novi Sad, Petrovaradin, Petrovaradinski Dunavac (al 2, ap 2, n 3); 10.09.2020. Bačka Palanka, Mladenovo, Lovrenac (ap 7, n 6); Bačko Novo Selo, Doktor pumpa (al 1, ap 2, n 11); Bačko Novo Selo, Hašaški Dunavac (al 13, ap 1, n 15); 11.09.2020. Apatin, Bara kod tri mosta (ap 1, n 3); Apatin, Markova bara (n 3); Apatin, Osmica (al 2, ap 10, n 37); Apatin, Tošina bara (al 1, ap 7, n 20) [DM]; 18.09.2020. Novi Sad, Begeč, Begečka jama (ap 5, n 12) [DC]

Schoenoplectus lacustris (L.) Palla, Cyperaceae: 09.09.2020. Novi Sad, Koviljski rit, Agla (al 1, ap 3, n 9) [DM].

Spirodella polyrhiza (L.) Schleid, Araceae: 09.09.2020. Novi Sad, Koviljski rit, Agla (ap 1, n 4); Novi Sad, Koviljski rit, Karlovački Dunavac (ap 1, n 3); Novi Sad, Koviljski rit, Rupa (al 2, ap 5, n 11); 10.09.2020. Bačka Palanka, Mladenovo, Lovrenac (n 2); Bačko Novo Selo, Hašaški Dunavac (ap 1, n 56); 11.09.2020. Apatin, Tuškoš (ap 2, n 3) [DM]; 18.08.2020. Novi Sad, Begeč, Begečka jama (ap 1, n 10) [DC].

Trapa natans L., Lythraceae: 09.09.2020. Novi Sad, Koviljski rit, Agla (ap 11, n 59); 10.09.2020. Bačko Novo Selo, Doktor pumpa (al 1, ap 1, n 4); 11.09.2020. Apatin, Markova bara (ap 8, n 22); Apatin, Osmica (n 10); Apatin, Tuškoš (al 1, ap 1, n 3) [DM].

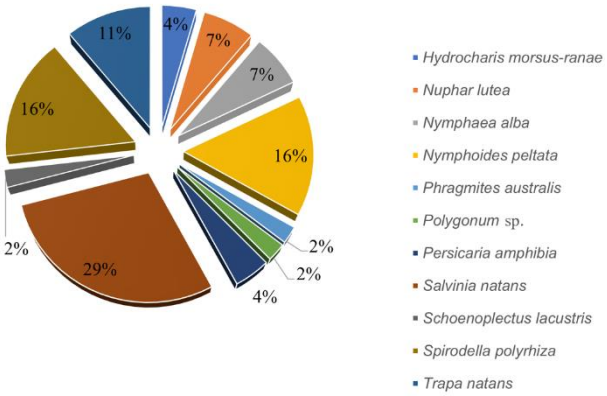

Fig. 3 Percentage of secondary hosts in the collected samples.

Discussion

During the research conducted in 2020, eleven new plant species were identified as secondary hosts for *R. nymphaeae* in Serbia. These plants are listed in the Host Plant Catalog of Aphids [8] as common hosts found in many countries in Europe and Asia. Our results have shown that the most common secondary hosts were *Salvinia natans* (present in 29% of all collected samples), *Nymphoides peltata* (16%), *Spirodella polyrhiza* (16%), and *Trapa natans* (11%) (Fig. 3).

The water lily aphid was found on more than 150 different host plants in Europe [8]. Belarus, Bulgaria, and Serbia reported two of the same primary hosts: *P. cerasifera* and *P. domestica* [7, 20]. In Bulgaria, water lily aphid was also found on *Amygdalus communis* L. and *P. armeniaca* [7, 21], while in Serbia it was reported on *P. spinosa* [9]. In Turkey, *R. nymphaeae* was seldom found on lettuce, *Lactuca sativa* (L.), where it can represent an important virus vector [22]. Water lily aphid is studied as one of the dominant pests of ornamental water plants in Hungary such as *Nymphaea alba*, *N. pubescens* Willd., *Nuphar lutea*, and *Nelumbo nucifera* Gaertn. [23], and other plants from the Nymphaeaceae family in Slovakia [24]. In our country, we found *R. nymphaeae* on two hosts from the Nymphaeaceae family (*Nymphea alba* and *Nuphar lutea),* and three other ornamental plants (*Nymphoides peltata, Persicaria amphibia,* and *Salvinia natans*). Water lily aphid also feeds on wild and semi-wild noxious water plants such as *Myriophyllum spicatum* L. in Russia [25] *Potamogeton* L. in Hungary [23], *and Trapa natans* in Italy, Hungary, Latvia, Romania, and Russia [9, 23, 25]. As for the wild plants, during this study in Serbia, it was found to feed only on *Trapa natans*.

Conclusion

Rhopalosiphum nymphaeae is a significant pest of ornamental water plants and various members of the Rosaceae family. However, as a reservoir of many parasitoid species, and pest of harmful wild and semi-wild water plants, it has the potential to be used in their biological control. While water lily aphid has a great number of hosts, it was found on only 19 different plants in Serbia. Future research should give us more information about water lily aphids' hosts, and their importance for aquatic and terrestrial ecosystems.

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Case Report

EWING SARCOMA FAMILY OF TUMORS OF SCAPULA IN CHILDREN

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Abstract. *Ewing's sarcoma (ES) is the second most common malignant bone tumor accounting for 5-10% of malignancies in childhood. The scapula is rarely affected and only several reports have been published. Early symptoms of Ewing's sarcoma of the scapula may be ignored by patients or misinterpreted by physicians, leading to a delay in diagnosis. In the retrospective analysis, we identified 3 patients (2 males) with Ewing's sarcoma of scapula, aged 11 - 14½ years in a 15-year period. The introduction of combined treatment with multimodal chemotherapy, surgical resection and/or radiation therapy has improved the survival of patients with Ewing's sarcoma significantly. Several options for resection of ES of scapula were described with generally unfavorable functional results. Two patients were treated with chemotherapy and resection of the scapula. The third patient is on chemotherapy. Two patients died after 2½ and 4 years. In children and adolescents presenting with shoulder pain, Ewing's sarcoma must be ruled out*.

Key words: *Ewing's sarcoma; PNET; scapula; children, diagnosis, treatment, bone cance*

Introduction

Ewing sarcoma of bone (ES), extraskeletal Ewing sarcoma, primitive neuroectodermal tumors of bone and soft tissue (PNET) and Askin tumor are malignant tumors known under the common name Ewing sarcoma family of tumors (ESFT) [1] because they share many common characteristics. Ewing sarcoma is the second most frequent malignant bone tumor in children and adolescents after osteosarcoma and accounts for about 5-10% of bone malignancy in childhood [2]. The reported incidence of ES is 2.5-3 cases per million per year [1, 3]. Slight male predominance has been reported [4]. Ewing sarcoma exhibits marked racial difference in incidence. It most frequently occurs in Caucasians [4, 5] and is uncommon in Black and Chinese children. Although ESFT may occur at any age the peak incidence is between 10 and 15 years, but almost 30% of patients are younger than 10 [6]. Occasionally ESFT may occur in children younger than 5 and in adults over 30 years [1, 7]. Most of the ESFT tumors arise in diaphyseal or meta-diaphyseal regions of long bones followed by the pelvis, ribs and spine [1, 8]. Other less frequent locations include the scull, scapula and small bones of hands and feet [2, 9]. In contrast to other malignant primary tumors of bone, about 20% of ESFT develop in soft tissues [1, 2]. The delay between onset of symptoms and definitive diagnosis is common [10]. At the time of the diagnosis 15-50 % of patients have distant metastases and 5 % have metastases in regional lymph nodes [4, 7, 11]. The most common sites of metastases are lungs, bones and bone marrow or a combination thereof. Primary bone tumors localized in the scapula are generally rare. They are more likely to be malignant than benign [12]. Tumors of the scapula usually grow significantly before the definitive diagnosis. In the beginning tumors are usually concealed within surrounding muscles. The early symptoms are often attributed to physical or sport activities, growth or trauma. In 2011 Shahid found less than 15 published cases of ES of scapula [12].

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The Aim

The aim of our study is to present three cases of ESFT in children with a primary location in the scapula.

Materials and Methods

We performed a retrospective database search for the patients diagnosed with ESFT of the scapula in the period 2001-2016. Three patients have met the inclusion criteria. Patients' data, imaging studies including ultrasound, x-rays and MRI, laboratory findings, histopathology reports, treatment and outcome of treatment were analyzed.

Results

In the fifteen-year period, ESFT of scapula was diagnosed in three patients, two boys and one girl. The mean age was 11.3 years. The right scapula was involved in two and the left in one patient.

A summary of the patients is given in Table 1.

Case #1

Eleven and a half years old boy was referred to pediatric surgeon by a pediatric hematologist. Initially, he presented to pediatrician with cervical lymphadenopathy several weeks earlier. When a palpable mass in his left scapular region had been discovered by a hematologist, he was referred to pediatric surgeon. On physical examination swelling in the left scapular region was obvious. The overlying skin was erythematous, without an increase of local temperature. The swelling was solid and painful on palpation. The mass was not mobile in relation to a deep underlying structures. Shoulder motion was unrestricted. There were palpable cervical lymph nodes along the left sternocleidomastoid muscle. The boy had no previous history of night pain or excessive sweating. His previous medical history was unaltered. His erythrocyte sedimentation rate (ESR)was elevated (42/72), but RBC ($4.15x10^6$/μL), WBC ($9.4x10^3$/μL) and hemoglobin (10.5g/dL) were within physiological ranges. Alkaline phosphatase ALP) level of 545 mmol/L was within normal range for his age (0,000-650 mmol/L), but lactate dehydrogenase (LDH) level was elevated to 510 U/L (normal range 200-400 U/L). All other biochemical tests were within normal ranges. The abdominal ultrasound was normal. An ultrasound of left shoulder region revealed solid lobulated inhomogeneous mass whose dimensions were 35 x 31 mm (Fig. 1). The tumor was located in soft tissues of suprascapular region, adjacent to bone. The mass had highly developed arterial and venous vascularization. Next to dorsal surface of tumor thin layer of fluid was visible. Computed tomography (CT) showed irregular, ovoid, inhomogeneous tumorous formation in left suprascapular region that extended to lateral border of acromion. Mass was hypo-dense with attenuation values of 42 HU. Acromion appeared osteoporotic, with destructed bone morphology. The tumor showed a relatively clear margin to surrounding soft tissues. After the application of contrast, intensive uptake was observed with an increase in attenuation values for approximately 10-15 HU. CT-scan was suggestive of a highly vascularized tumor of the left scapular region. The

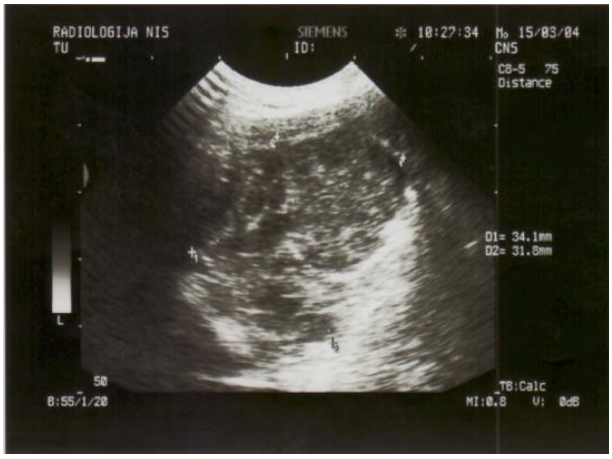

Fig. 1 Inhomogeneous, ill-defined, ultrasound appearance of ES of scapula (Case 1) measuring 34 x 31 mm.

patient was prepared and operated on. Resection of tumor along with the whole acromion and part of the scapula was performed with wide margins. Resected tumor measured 7 x 4 x 3.5 cm. On histology tumor was composed of small dense uniform round cells with round nuclei and scarce cytoplasm and the histopathology diagnosis was Ewing's sarcoma. Chest x-ray and CT scan didn't show pulmonary metastasis. The patient was then referred to the national oncology center where he received multimodal adjuvant chemotherapy. He was doing well for three years postoperatively. Then pulmonary metastases have been detected and he succumbed four years after surgery.

Case #2

A fourteen-and-a-half-year-old girl presented with swelling and pain in her right shoulder. Four months earlier she had begun to feel pain in shoulder region. The pain was intermittent and present during day only. She went to a primary care physician and was referred to physiotherapy. During the manual massage which was very painful mild swelling in the right scapular region was noticed. Physiotherapy was discontinued and she was prescribed rest, oral and topical analgesics. Sine the pain became constant, with the increased intensity especially during the night she was referred to a pediatric surgeon. On the initial clinical examination the swelling of the right scapular region was observed. The overlying skin looked normal. On palpation the tumor appeared solid and poorly defined from surrounding soft tissue. The mass was painful on palpation with no mobility in relation to underling structures or overlaying skin. The ultrasound revealed poorly defined, inhomogeneous mass within the soft tissue of scapular region (Fig. 2A). On plain X-ray the bone structure of right scapula appeared irregular and mottled (Fig. 2B). The patient was admitted for further laboratory tests and MRI. The blood count was normal, but ESR was elevated (57/85). In blood biochemistry elevated levels of alkaline phosphatase (722 mmol/L; normal range 0,000-650 mmol/L) and lactate dehydrogenase (658 U/L; normal range 200-400 U/L) were observed. Other parameters including liver and renal function were within normal ranges for age. MRI revealed expansive tumor formation which had infiltrated body and spine of right scapula also infiltrated supraspinatus, infraspinatus and subscapularis muscles (Fig. 2C-F). No infiltration of chest wall was demonstrated.

Open biopsy of the tumor was performed. The histology confirmed tumor composed of small round cells with round nuclei and one nucleolus with a very small cytoplasm. The diagnosis of Ewing's sarcoma had been established and confirmed by immunologic studies performed in another laboratory. Disseminated pulmonary metastases were noticed on chest CT scan. The patient was referred to the national oncology center for multimodal chemotherapy protocol. After 6 cycles of neoadjuvant chemotherapy scapulectomy was performed followed with postoperative chemotherapy. She died 2½ year after the diagnosis.

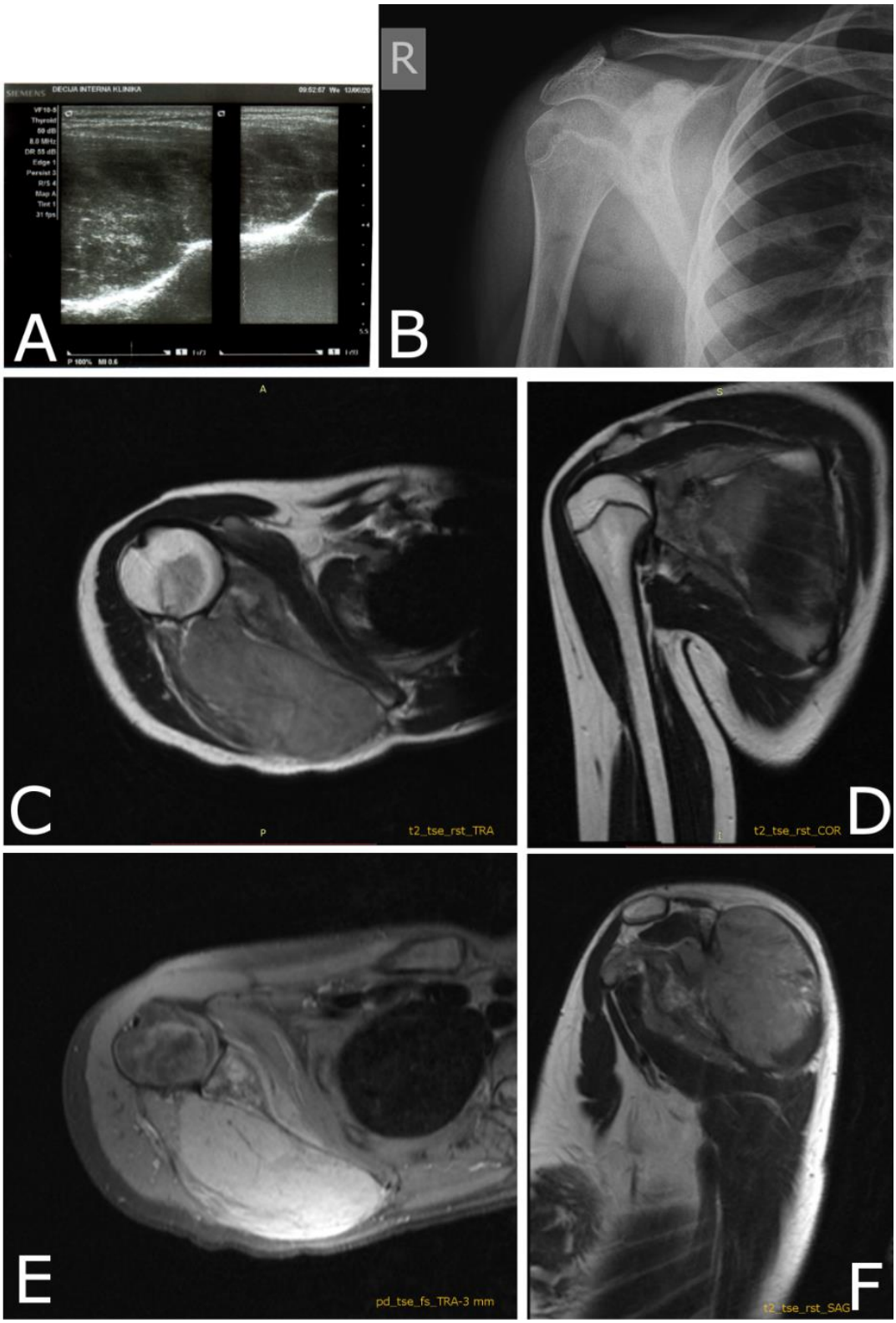

Fig. 2 A) Inhomogeneous mass on ultrasound of right scapular region (Case 2). B) Plain X-ray of right scapula with permeative lesions without sclerosis. C-F) MRI findings of the same patient. C) Large soft tissue mass on transverse section. D) Infiltration of body of scapula and subscapularis muscle on coronal section. E) Bone and muscular infiltration on transverse f-stir section and F) sagittal section.

Case #3

An eleven year old boy was referred from the community regional hospital on an emergency basis because of suspected phlegmon in the right scapular region. A month earlier, after minor trauma (he was hit by another boy during the school skirmish) he felt pain in the right shoulder. The swelling occurred on the next day. The diagnosis of contusion was established by a primary care physician. Rest, topical analgesics and oral painkillers were prescribed. Nevertheless, swelling and pain increased boy became febrile, and he was admitted to the local pediatric department where he was treated with IV antibiotics for 4 days. His condition had worsened despite therapy and he was transferred to a tertiary level institution. On admission, the patient was sub-febrile (37.8^0C) and complained of severe pain. Clinically, swelling in the right scapular region was obvious. The skin was erythematous with increased local temperature. Movements in the right shoulder were painful and reduced to about half of the normal range. The erythrocyte sedimentation rate was elevated to 40/80. Blood tests showed normal levels of WBC and RBC, a slight decrease in hemoglobin level (119g/L) and thrombocytosis of 424 x 10^9/L. Levels of ALP and LDH were within normal ranges (152 U/L and 383U/L respectively). Other biochemical parameters were normal, except the elevated level of C-reactive protein (112.05 mg/L, normal range 0.00-0.5). The abdominal ultrasound was normal. On ultrasound examination of thte right scapular region two inhomogeneous, poorly delineated, expansive masses approximately 19 x 9 mm and 35 x 20 mm in diameter were detected within muscles (Fig. 3A). Color Doppler revealed intense peripheral vascularization of tumor tissue. On plain x-ray's right scapula appeared inhomogeneous, with permeative bone destruction, especially along the superior half of the medial border and infraspinous fossa (Fig. 3B). MRI of the right shoulder region was performed. An expansive tumor that infiltrated the body, spine and part of the acromion of the right scapula was found. Supraspinatus, infraspinatus, subscapularis and trapezius muscles also were infiltrated. After the application of contrast an intense, inhomogeneous increase in signal occurred (Fig.3C and D). Numerous lymph nodes were detected in the suprascapular region and in the right axilla. No in-

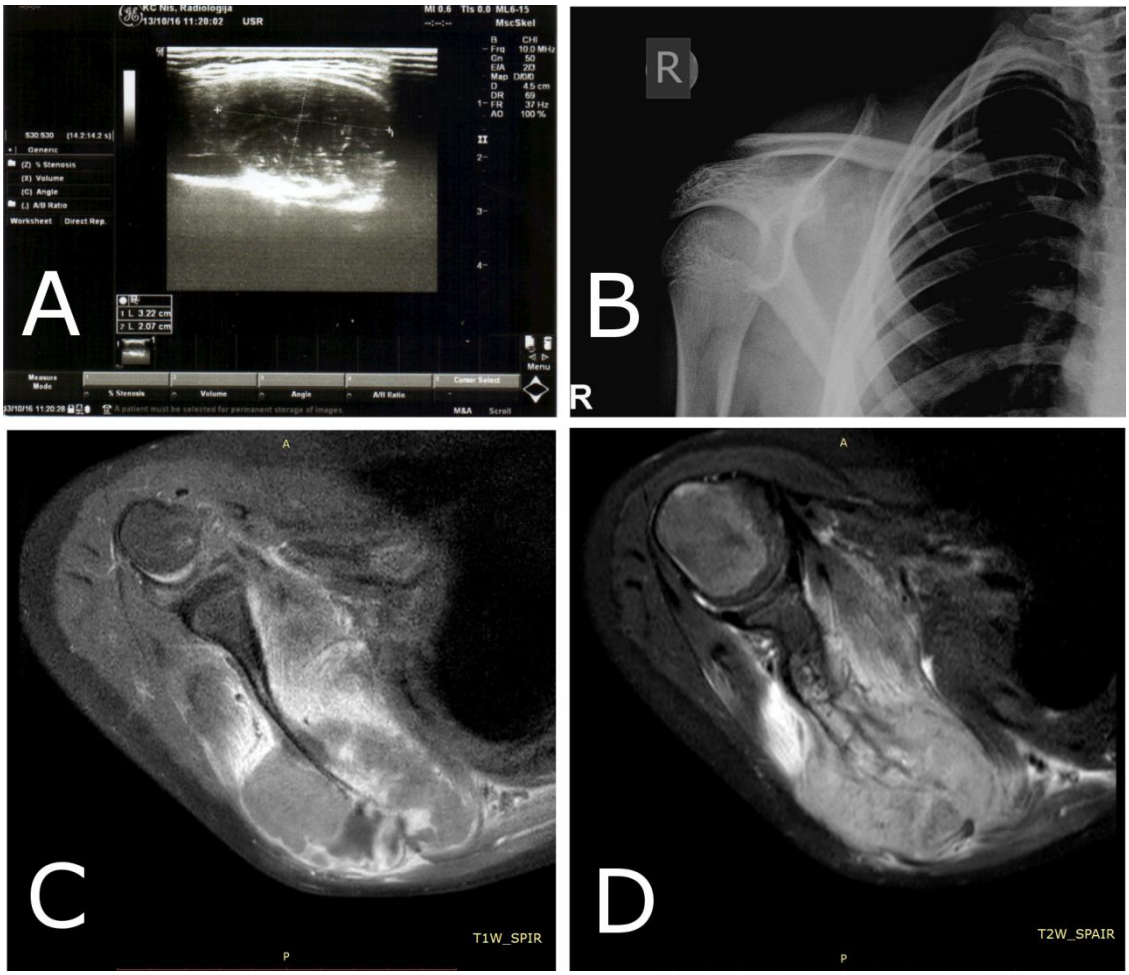

Fig. 3 A) Inhomogeneous ultrasound appearance of ESFT (Case 3.) B) Plain X-ray shows irregular, moth-eaten bone structure of right scapula in upper and medial part. C) Tumor infiltrating subscapularis and supraspinatus muscle is visible on transverse MRI T1W section. D) Infiltration of body of scapula and muscles by tumor is noticeable on transverse T2W MRI sequence.

Table 1 Patients overview.

	Case 1	Case 2	Case 3
Time from onset of symptoms to diagnosis	4 weeks	4.5 months	4 weeks
Leading symptom	Cervical liymphadenomegaly	Pain	Swelling and pain
Laboratory findings			
ESR*	Elevated (42/72)	Elevated (57/85)	Elevated (40/80)
ALP#	Normal (545)	Elevated (722)	Normal (152)
LDH$	Elevated (510)	Elevated (658)	Normal (383)
Histopathology	Ewing sarcoma	Ewing Sarcoma	ESFT PNET
Immunohistochemistry		$CD99^+$, $Vimentin^+$ $FLI1^+$	$CD99^+$, $Vimentin^+$ $FLI1^+$ Synaptophysin $^{+/-}$ $CD56^{+/-}$
Metastases at presentation	No	Yes, pulmonary	No
Preoperative chemotherapy	No	6 cycles	4 cycles
Surgery	Wide resection	Scapulectomy	-
Survival	4 years	2.5 years	Lost for follow/up

*ESR – erythrocyte sedimentation rate
#ALP – alkaline phosphatase
$LDH – lactate dehydrogenase

volvement of chest structures, humerus and clavicle has been observed. Open biopsy and bone marrow aspiration was performed under general anesthesia. Histopathology showed tumor composed of small, round, hyperchromatic cells with scant cytoplasm and one nucleolus in a round nucleus. Tumor cells were organized in sheets with the focal formation of pseudo-rosettes. Immunohistochemistry demonstrated cells positive on CD99, vimentin, and FLI-1, partially positive on synaptophysin and CD56 and negative on desmin, myogenin chromatogranin and LCA. Bone marrow aspirates were normal. No pulmonary metastases were noted on chest CT scans. The boy was referred to the national oncology center for chemotherapy protocol. He received four cycles of chemotherapy until he was lost for further follow-up.

Discussion

Ewing sarcoma is highly malignant primary bone tumor, composed of uniform round small cells with round nuclei and scant cytoplasm Tumor cells produce no matrix. Cytoplasm is (PAS+) [2] because of glycogen content. Ewing sarcoma is frequently associated with translocations resulting in fusion transcript EWS-FLI [13] or EWS-ERG [14]. This is crucial in tumorigenesis, transdifferentiation into characteristic small round cell phenotype and neural marker expression [2]. This common genetic profile of several small round cell tumors is now grouped under entity Ewing Sarcoma family of tumors – ESFT with poorly differentiated ES on one and more differentiated PNET lying on the other end. Theis family of tumors shares the same neurogenic cell of origin as was demonstrated on electron microscopic and immunohistochemical studies [4]. Two tumors in our series exhibit typical histological features of small round cell ES and the third tumor demonstrates signs of neurological differentiation characteristic for PNET. Immunohistochemical positivity for CD99, vimentin and FLI1 is detected in majority of ESFT. In more differentiated PNET tumors positivity for neuron-specific enolase (NSE), synaptophysin, S-100, Leu-7(CD57) and/or PGP 9.5 may be demonstrated [2, 4, 10]. Two of our cases were positive for CD99, vimentin and FLI1, and one of them also showed partial immunoreactivity for synaptophysin and CD56. For the third case we could not obtain immunohistochemistry data.

Peek incidence of ESFT in the second decade of life coincides with an increase in the secretion of insulin-like growth factor 1 (IGF-1) and the sensitivity to IGF-1 receptor inhibition is the hallmark of ESFT. All our patients were in the first half of the second decade of life at the presentation.

The majority of ESFT arises in long bones of the lower extremity, pelvis, chest wall and spine [1] but up to 20% may develop in soft tissue. Malignant tumors of the scapula are generally rare and include chondrosarcoma, synovial sarcoma, Ewing sarcoma (ES) and metastasis [15]. Reports of ESFT of the scapula are scarce [12, 15-18]. The earliest symptom of ESFT in the majority of patients is pain, mild and intermittent in the beginning but with a gradual increase in intensity over time. The onset of night pain should always be considered as a warning sign that requires further investigation [8]. The pain localized in the scapula may be erroneously attributed to various conditions like overuse syndrome, growth-related pain, or trauma in patients with ESFT. Our patients had mild pain at early stages which increased over time. In all of them the pain was initially attributed to different causes such as cervical lymphadenomegaly in the first, overuse in the second and trauma in the third patient. In the second and third patient the pain was of such intensity at presentation that it could not be relieved by oral analgesics. ESFT typically grows fast and may become quite

large within several weeks. In specific locations, where tumor is confined within muscles, covered with large soft tissue layer or it grows in cancellous bone or medullary canal without penetrating the cortex [19] it may take several months or even year until it becomes visible or palpable . The interval from the onset of symptoms to the diagnosis of ESFT decreased from 9.6 months in 1984 to 4.7 months in 2003 [4] but delay in the diagnosis of 3 to 6 months is not uncommon. In two of our patients duration of symptoms was relatively short (6 and 4 weeks in cases 1 and 3 respectively) but it could be shortened for 2-3 weeks if bone tumor had been included in differential diagnoses. In the third patient, (case 2) the time from the onset of symptoms to the moment of establishing the diagnosis was 4.5 months, because her symptoms were attributed to the overuse syndrome, despite the night pain. The delay in establishing the diagnosis could be doctor-related – the wrong diagnosis on presentation based on clinical or ultrasound findings; or patient related – usually painless mass that is ignored [20]. Although the diagnosis of ESFT in our patients was established within expected time frame, every effort should be made in order to maximally reduce unnecessary time loss in those patients. Some patients with ES may have fever. Only one patient in our group had fever (case 3), initially misinterpreted as a consequence of infection of the posttraumatic hematoma. The erythrocyte sedimentation rate is usually elevated, as was the case in all our patients. The patients with ES also may have anemia and leukocytosis [4, 19] but these parameters were normal in our group of patients. High values of ALP and LDH may be observed. Elevation of their levels is the unfavorable prognostic factor, as was in the first two of our cases.

The initial imaging study should be plain X-rays. In long bones, ES has typical radiologic picture of an ill-defined diaphyseal and/or metaphyseal destructive lesion with onion skin-like appearance and periosteal reaction but in flat bones of pelvis or scapula identification of ES could be difficult [4]. Ewing's sarcoma of scapula usually has a permeative, moth-eaten appearance with little or no bone sclerosis on plain X-rays [12] which was clearly visible in our patients (Fig. 2B and 3B). The next step in the imaging should be MRI [19]. On MRI Ewing's sarcoma appears as an extraskeletal mass derived from bone that exhibits low signal intensity on T1-W and high signal intensity on T2-W sequences [4]. If MRI is contraindicated CT should be performed. For the detection of pulmonary metastases, CT scans are the most appropriate imaging modality. For the detection of skeletal metastases, whole body scintigraphy is often used. Recently, it has been demonstrated that F-18-deoxy-d-glucose positron emission tomography (18FDG-PET) may be sufficient for initial detection of bone metastases and bone marrow involvement [21]. In the first patient CT scan was performed because of temporary unavailability of MRI. In the other two patients (cases 2 and 3), MRI showed tumor that infiltrated scapula with large soft tissue mass expanding in subscapularis, supra- and infraspinatus muscles (Fig. 2C-F and Fig. 3C&D). Ultrasound may have misleading role in diagnostics of ESFT. The primary value of US is in visualization of soft tissue mass (Fig. 1A, 2A and 3A), and if it appears as highly vascularized lesion on color Doppler the patient should be referred to the institution accredited for the treatment of such tumors without any delay [8]. Abdominal ultrasound is recommended as a part of full diagnostic workup.

In all 3 children, the open incisional biopsy was performed for adequate tissue sampling [19], although fine needle aspiration biopsy and core needle biopsy may be performed as well [2]. Modern treatment of ESFT includes multimodal systemic chemotherapy and local control of tumor by surgery and/or radiotherapy [8]. The role of preoperative neoadjuvant chemotherapy is to eradicate distant micrometastases and reduce tumor volume to facilitate its resection [4]. Although amputation was the only surgical option for decades, current surgical management of ESFT includes limb salvage procedures with tumor resection and reconstruction [19]. Wide margins should be the goal of surgical resection in ESFT as is in all other high-grade malignancies. Surgical options for malignant bone tumors of the scapula include total scapulectomy, constrained prosthesis and allograft [17]. Subtotal scapulectomy with glenothoracic fusion has been developed to improve shoulder function [22]. Hoornenborg et al. recently described the treatment of ES of the scapula in a 9½ year-old boy with resection, extracorporeal irradiation and re-implantation with good results [15]. Once radiation therapy was the primary method of local control. Currently, it plays a role in patients with inoperable tumors, tumors that were resected within unsafe margins or if the response to chemotherapy was inadequate [4]. When the possibility of achievement of wide margins is uncertain, preoperative irradiation may be added to the treatment [19]. Wide resection of tumor was accomplished by subtotal scapulectomy in one of our patients (case 1). He didn't receive preoperative chemotherapy. In the second patient (case 2), after 6 cycles of preoperative neoadjuvant chemotherapy scapulectomy was performed. The third patient had received 4 cycles of chemotherapy before he was lost for follow- up.

Several factors are associated with unfavorable prognosis of ESFT: location in axial skeleton, size of tumor > 8 cm, elevated serum LDH, patients older than 14 years, inadequate or no surgical resection of the tumor and poor response to preoperative chemotherapy, but the presence of metastatic disease at the time of diagnosis remains strongest adverse prognostic factor [1, 8]. The five-year survival for ESFT patients treated with surgery or radiation alone is less than 10%. The adjunct of aggressive multimodal chemotherapy improved the 5-year survival to 60-70% in patients with localized and 20-40% in cases of metastatic disease [4, 19]. Both patients with poor outcome in our study had elevated LDH, large tumors (7 and 9.5 cm) and unfavorable location of tumor in scapula. One patient was older than 14 years and had pulmonary metastases and the another one had tumor resection without preoperative chemotherapy. The third patient has no adverse prognostic factors.

In the children in the second decade of life presenting with the pain in the scapular region, the intensity of which gradually increases and especially if it appears overnight, ESFT should always be considered as a potential diagnose. Visible and palpable mass may appear later. Elevated ESR, ALP and LDH are common finding. Ultrasound is usually misleading. Although a moth-eaten appearance of the scapula is characteristic X-ray finding, MRI is necessary for establishing the diagnosis. Histopathology and immunohistochemical analysis of biopsy material confirms diagnosis of ESFT. All patients with suspected ESFT od scapula should be referred to the appropriate center for further diagnosis and therapy without any delay. Neoadjuvant chemotherapy along with surgical resection and radiation therapy in selected cases increases the chance of survival. Metastatic disease at the time of diagnosis remains the most important prognostic factor for the unfavorable outcome.

Conclusion

Ewing's sarcoma family of tumors of the scapula is very rare in children and only several cases have been described in the literature so far. Tumor confined deep within muscles around the scapula may grow significantly before the onset of symptoms. Furthermore, symptoms may be ignored by patients or misinterpreted by doctors, and attributed to benign conditions such as overuse syndrome, trauma or infection, leading to a delay in diagnosis and an increase in the risk for the development of metastasis. Anyone to whom a young child or adolescent presents with the pain in the shoulder should be aware of the possibility that ESFT may be the cause of symptoms, especially if the pain is constant, and appears at night. The best option is to refer such a patient to a specialized center where a complete diagnostic workup could be promptly accomplished. Early diagnosis, current multimodal chemotherapy accompanied with surgical resection and/or radiation therapy may improve the prognosis of the patients with ESFT of the scapula. Limb salvage and reconstructive procedures to preserve shoulder function should be preferred surgical options whenever possible.

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Review Article

A SUGGESTION FOR PROACTIVE CARDIOLOGIC APPROACH TO CUSHING'S SYNDROME OR DISEASE

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Abstract. *Numerous studies and reviews agree about the increased cardiovascular risk in Cushing's syn-drome. Therefore, the aim of the paper is to suggest a few common diagnostic and therapeutic cardiologic preferences for the majority of Cushing's syndrome/Cushing's disease (CS/CD) pa-tients which are not yet routine but have the rationale to become standard procedures. This may serve as an initial working document, to be improved by the experts in the field. A narrative review is used to present synthesis and deduction of several approaches in cardiology regarding the actu-al topic. Results are systematized as the risk factors or co-morbidities list (prevalent in CS/CD) coupled with current and adapted cardiologic suggestions for practice.*

Key words: *Cushing's syndrome, Cushing's disease, ACE inhibitor, spironolactone, statin, holter monitoring*

Introduction

Cushing's syndrome (CS) is important due to the high prevalence [1] of its exogenous (mostly iatrogenic) form [2,3]. Numerous comorbidities are often present in CS / Cushing's disease (CS/CD) [4,5]. The mortality rates are increased in CS/CD [4,6] in the range between 1.8 and 7.4-fold higher [5]. Numerous studies and reviews agree about the increased cardiovascular risk in CS/CD [2,4-6]. Cardiovascular risks ought to be repeatedly estimated in clinical practice and care should be taken to control them optimally, because they are pronounced and persistent -it is not easy to eliminate hypercortisolism and even if it is achieved- cardiovascular risk factors may still be present [7].

Unfortunately, abundant evidence of high cardiovascular risk in CS/CD is sub-optimally translated into practical recommendations [5]. There is a step between recognizing increased risk in the medical literature and incorporating this knowledge into the physicians' usual care for the patient. The cardiologic part of such interdisciplinary recommendations (endocrinologic and cardiologic) is largely missing [5].

Therefore, the *aim* of the paper is to suggest a few common diagnostic and therapeutic cardiologic preferences for the majority of CS/CD patients which are not yet routine but have the rationale to become standard procedures. This may serve as an initial working document, to be improved by the experts in the field.

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Materials and Methods

A narrative review is used to present the synthesis and deduction of several approaches in cardiology regarding the actual topic. We performed a search in the following databases: Medline, Springer, Elsevier, SAGE, Oxford Press, Wiley, and the search engine Google Scholar. Results are systematized as the risk factors or co-morbidities list (prevalent in CS/CD) coupled with current and adapted cardiologic suggestions for practice.

Results

Arterial hypertension (HTN) (Table 1) is very prevalent in CS/CD in the range of 70% to 90% [6,5]. Blood pressure (BP) may increase early in CS/CD. For example, HTN starts during the first day of oral intake of 80 mg – 200 mg cortisol daily and peak BP increase occurs after 4 or 5 days [8]. Ambulatory BP monitoring (ABPM) is now very important for HTN detection and evaluation [9-11]. ABPM can be recommended for many CS/CD patients, particularly if long-standing and/or severe [12]. Importantly, ABPM can be used to detect masked arterial hypertension (MAHT) [9-11] in CS/CD patients because

they have numerous characteristics associated with MAHT [12]. Additionally, there is a place of ABPM in CS/CD patients with sustained HTN – to evaluate antihypertensive therapy [9].

Obesity and DM increase the likelihood of uncontrolled or masked uncontrolled HTN (MUCH) [9] and they are features of CS/CD [5]. Moreover, in CS/CD there is often persistent hypercortisolism, which promotes HTN [7]. In the pathophysiology of HTN in CS/CD numerous factors play a role: obesity, elevated cardiac output, activation of the renin-angiotensin-aldosterone system (RAAS), increased cardiovascular sensitivity to vasopressin, angiotensin II and catecholamines, reduced efficacy of vasodilatory mechanisms, higher total peripheral vascular resistance, mineralocorticoid action of cortisol and mineralocorticoid hormones co-secretion (e.g., deoxycorticosterone), sleep apnoea, etc[5,7,8,13-15].

RAAS blocker (ACE inhibitor or angiotensin receptor blocker (ARB)) is one of the first choices for HTN treatment in CS/CD [15,16]. RAAS blocker is generally a part of the preferred combination to start antihypertensive therapy with [9] RAAS blocker is also indicated in obese HTN patients [9] and most of the patients with CS/CD are obese [5].

In addition to the RAAS blocker, a direct suggestion for antihypertensive therapy in CS/CD patients is a *mineralocorticoid receptor antagonist (MRA)* [16]. One of the reasons for this recommendation is the known characteristic of hypercortisolism (particularly if severe) that the kidney enzyme 11b-hydroxysteroid dehydrogenase type 2 cannot convert all cortisol to cortisone. As a result, an excessive amount of cortisol binds to the mineralocorticoid receptor and produces the effects of mineralocorticoid surplus, including salt and water accumulation with BP increase and K^+ decrease [13]. Therefore spironolactone or e.g. eplerenone can be recommended for patients with CS/CD. MRA spironolactone is expected to be advantageous [14] due to excessive action of both glucocorticoids and mineralocorticoids "non-selective" CCBs are also candidates for CS/CD patients [16] because they are an essential part of the most triple antihypertensive therapies [9].

The additional reason to consider *CCBs* in CS/CD patients is that short-term variability of BP is higher in CS patients [17] and it is known that CCBs and ACE inhibitors diminish visit-to-visit BP variations [18] particularly if they are obese and their HTN is severe. The escalation of antihypertensive treatment is to be expected, because HTN was controlled in only 15% of CS patients [19].

In addition to its own significance *dyslipidemia* is an important problem in CS/CD patients because it is prevalent and clustered with other risk factors of atherosclerosis [5]. Moreover, this association between risk factors is quantitative, e.g., LDL-cholesterol is significantly and independently associated with systolic BP in CS/CD [38]. A common pattern of dyslipidemia in CS/CD is represented by the increase in LDL-cholesterol and triglycerides and decrease of HDL-cholesterol [39].

Chronic hypercortisolemia leads to insulin resistance; therefore CS/CD is the metabolic syndrome's archetype. Overweight or obesity is found in > 50% of CS/CD patients and DM is also very prevalent (up to 50%) [40]. The obesity and DM, prevalent characteristics of CS/CD patients, make hyperlipidemia more difficult to control [41]. It is particularly so if hypercortisolism is not controlled. Even following the effective therapy of CD (despite a decrease of BP and body mass index) the majority

Table 1 Some cardiologic drugs and diagnostic procedures we suggest for more regular use in CS/CD patients

Comorbidity /Characteristic of CS/CD	Reference confirming that this comorbidity /characteristic is prevalent in CS/CD	Drug (or diagnostic procedure) suggested for PRIMARY prevention /EARLY treatment (or early diagnosis)	Reference confirming the rationale for the cited drug (or diagnostic procedure) for particular co-morbidity /characteristic
HTN (early treatment)	[5,6]	RAAS blocker, spironolactone, consider timely (using ABPM) CCB, diuretic and BB	[15,16]
Hyperlipidemia (early treatment)	[5]	depending upon FHS or SCORE, consider statin	[20-22]
HF (primary prevention)	[5,23]	RAAS blocker, spironolactone	[24,25]
CAD (early diagnosis)	[5,26,27]	Pretest probability of CAD, ECG, exercise test, CT calcium score	[28,29]
CAD (primary prevention)	[5,26,30]	Consider aspirin and statin	[29,31]
Hypokalemia (early treatment)	[32]	spironolactone, RAAS blocker	[33,34]
VTE (early diagnosis)	[35]	Clinical prediction rule, D dimer, venous ultrasound	[36,37]

Legend: CS/CD – Cushing's syndrome/Cushing's disease; HTN – arterial hypertension; RAAS – renin-angiotensin-aldosterone system; ABPM – ambulatory blood pressure monitoring; CCB - calcium channel blocker; BB – beta-blocker; FHS – Framingham Risk Score; SCORE – Systematic COronary Risk Evaluation; HF – heart failure; CAD – coronary artery disease; CT – computerized tomography; ECG – electrocardiogram.

of patients (56%-76%) one year later still had obesity, HTN, DM, high cholesterol, and triglyceride levels [40].

In line with this, obesity (especially visceral) often continues even after surgical remission in CS/CD patients [41]; it may contribute to the persistence of hypercholesterolemia and hypertriglyceridemia [39]. This persistence of dyslipidemia can result from the continuation of increased BMI [41].

Unfortunately, some drugs used to suppress adrenal glands in CS/CD have unwanted effects in worsening dyslipidemia [41,42], which questions their clinical benefit. The significance of dyslipidemia and its association with other risk factors of atherosclerosis leads to the suggestion for its aggressive therapy [42]. Therefore a scene is set for treating hyperlipidemia in CS/CD. On an individual basis, eligibility for treatment can be estimated using the patient's Framingham Risk Score (FHS) or Systematic COronary Risk Evaluation (SCORE) [20-22] to evaluate the risk of CAD and mortality (and the need for antihyperlipidemic therapy). As in most other patients, for these with CS/CD in the first place, a statin ought to be considered.

The caution is advised because of *steroid myopathy* which is often present in CS/CD [43]. Proximal myopathy is regarded as additionally suggestive of CS (similarly to purple striae) [43,44]. It is a toxic non-inflammatory myopathy that dominantly affects pelvic muscles with consequent difficulties to stand up and climb up [45]. Steroid myopathy is the most prevalent among drug-induced myopathies; its incidence is around half of corticosteroid-using patients for prolonged periods [45]. The diagnostic approach is not standardized, and it is not easy to quantify the changes and to follow-up such patients adequately [43]. In addition to muscle symptoms (i.e. myalgia), weakness of proximal muscles can complicate statin use [45,46]. Unfortunately, muscle strength testing is not frequently performed; reports demonstrated muscle weakness in >10% of statin users [46]. Therefore, the likelihood of an indication for statin is substantial in CS/CD patients, but the follow-up ought to be adequate, particularly as far as steroid myopathy and hepatic lesions are concerned.

The risk of *HF* is increased in CS/CD patients, up to 6-fold [32]. The most important risk factors of HF are clustered in CS/CD, such as HTN, CAD, obesity, and DM [5]. Moreover, a direct effect of glucocorticoid excess upon cardiomyocytes is probable [23,47-49]. *An echocardiogram* is needed in CS/CD patients to evaluate the presence of structural and functional abnormalities of the heart, which are common in CS/CD patients, starting from left ventricular hypertrophy (LVH) as a result of several aforementioned risk factors. The echocardiogram is obviously indicated, and it is hopefully done in most CS/CD patients.

RAAS blocker and potassium sparring diuretic (spironolactone in the first place) can be recommended for *HF* primary prevention in CS/CD patients with HTN. In CS/CD patients with HTN and "borderline" HF (incipient symptoms with minimal NT pro-BNP elevation): in the first line diuretic *(MRA), RAAS blocker, and certain BBs (bisoprolol, carvedilol, metoprolol succinate [50] and nebivolol [25])* can be recommended. An adequate choice of certain BBs is needed because they are a very different class of drugs [51]. Also SGLT2 inhibitors can be suggested for evaluation in CS/CD patients.

Cardiovascular diseases are the main cause of death in CS/CD patients [52]. Therefore, it is reasonable to look for *CAD* in many CS/CD patients. Their age, symptoms, and risk factors can help us estimate the risk [28]. It is documented that CAD risk in many CS/CD patients is high or very high [26]. Moreover, CAD is more prevalent in CS/CD vs. controls (general population) up to 17 times [27].

In addition to the estimation of CAD probability for early diagnosis, several methods are widely available including ECG, exercise test, coronary artery calcium score, etc. Moreover, Holter is needed for arrhythmia and ischemia detection, as the risk of atrial fibrillation is also increased in CS/CD [32]. Indeed, for the estimation of AF risk, various risk scores can be useful [53]. In CS/CD at high risk of CAD we ought to consider ASA [29] and statin [20-22] in the primary prevention. In CS/CD patients with HTN at high risk of CAD, there is a rationale to consider RAAS blocker, and CCB or BB [9].

For *early detection of VTE*, one should recognize elevated risk in CS/CD patients. It is reasonable to use clinical prediction rules, D dimer, and venous ultrasound more liberally and more frequently in patients with CS/CD vs. without it.

To sum up, it is important that the clustering of cardiovascular risk factors in CD and CS due to ongoing long-term administration of high-dose glucocorticoid treatment is estimated by FRS and SCORE as high or very high [5]. Therefore, the cardiologic approach is typical for patients at high risk of CAD, with additional attention on more regular use of holter and ABPM.

Conclusion

The risk of cardiovascular events is high enough or will be high enough in the majority of CS/CD patients to warrant a cardiologic work-up. Due to scarce data on the individual benefit of examinations and tests more studies are needed to explore the cost-effectiveness of several cardiologic diagnostic and therapeutic procedures.

To our opinion, the vast majority of CS/CD patients ought to be evaluated as candidates for Holter and 24h ABPM in addition to echocardiography, due to the increased risk of numerous cardiovascular diseases. Aspirin, RAAS blocker, MRA, and statin may be subjects of the individual evaluation for the primary cardiologic prevention of CAD and HF in CS/CD patients.

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