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BIOLOGICAL EFFECTS OF A LOW-FREQUENCY ELECTROMAGNETIC FIELD ON YEAST CELLS OF THE GENUS *SACCHAROMYCES CEREVISIAE*

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Abstract

Background: Although the scientific community is extensively concerned with the effects of the EMF, the unambiguous explanation of its effects on living structures is still lacking.

Goals: The goal of the study was to evaluate the effect of a low-frequency (LF) electromagnetic field (EMF) on the growth and multiplication of the yeast *Saccharomyces cerevisiae*.

Methods: Yeast cells were exposed to a frequency of 900 Hz and a magnetic flux density of 2.3 mT. The duration of each experiment was 8 hours, in the beginning of the measurement the value of frequency, rms (root mean square) value of electric current (2 A), and magnetic flux density were fixed set on the exposure device. A paired experiment was performed, a sample exposed to EMF, and a sample shielded from the field. Subsequently, samples were taken every two hours, the number of cells was recorded, and then the concentration of the yeast cells was evaluated at time points. The time points reflected the exposure time of the samples exposed to EMF.

Results: The results indicate that LF EMF at given parameters has an inhibitory effect on the growth and multiplication of yeast cells.

Conclusion: Exposure to EMF can cause the differences in growth dynamics between cells exposed to the field and the unexposed ones.

KEYWORDS: non-ionizing radiation, electromagnetic field, low frequencies, biological effects, yeast.

INTRODUCTION

Non-ionizing radiation has been a topic of interest to the scientific community and the general public for decades. The number of sources of EM radiation has been constantly increasing (e.g. development and use of 5G networks), which raises the need for more extensive research in this area. Non-ionizing radiation does not have the ability to ionize atoms and molecules in the environment, and it is in the frequency range of 0 Hz to 10^{15} Hz. Artificial sources are all electrical appliances in households, factories, mobile phones, IT equipment, and electrical wiring. Extremely LF electric and magnetic fields are in the range 0 – 300 Hz and are emitted from electrical wiring, lamps, and appliances (1).

In addition to thermal effects, non-thermal effects can also occur. Non-thermal effects interfere with the function of the cardiovascular, nervous, and endocrine systems and excretory mechanism. According to some authors, human body can respond with some symptoms of the flu, headaches, fatigue, loss of concentration, behavioral changes, or liver cell degeneration (2-5). The effect of EMF was confirmed in 26 studies (Pall, 2016) that

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addressed the microwave syndrome which represents the neuropsychiatric effects caused by LF radiation (6). Microwave syndrome, currently known as electromagnetic hypersensitivity (EHS), is a set of multi-organ symptoms that occur upon a single intense or continuous exposure to EMFs. Biological effects have been demonstrated at the molecular level with extremely low intensity of magnetic and radio-frequency (RF) EMFs (7). More than 220 scientists point out that EMFs affect biological structures even at lower intensities than those set out in most international guidelines. The effects include an increased risk of cancer, cellular stress, increased levels of harmful free radicals, genetic damage, structural and functional changes in the reproductive system, learning and memory deficits, neurological disorders, and a negative impact on human well-being (8). The study by Misek et al. (2018) points the need to monitor each new base transceiver station which is a source of low energy but permanent whole-body exposure (9). Blank and Goodman (2009) argue that EMF has the ability to act on human cells and DNA and the cells respond by a stress response (11). Presence of stress proteins is a sign that the cell has encountered something harmful and this situation also occurs when interacting with EMF in the whole EM spectrum. According to Blank and Goodman (2011), DNA seems to operate as a fractal antenna (an antenna that amplifies the frequency range) and, thus, responds to different frequencies. Also, it was found that EMF can disrupt an integrity of DNA, blood cells, nerve cells, or affect arterial blood pressure and pulse rate (10-12). According to Jakusova et al. (2010), the duration of a mobile phone call is related to a burning sensation in the ear area, loss of concentration, and sleepiness (13). The authors also show the particular types of personal protection (14). In another study, EMF with a frequency of 50 Hz can increase the expression level of yeast genes involved in cell metabolism (15). Novak et al. (2007) also confirmed that the number and growth of yeast of the genus *Saccharomyces cerevisiae* were reduced upon exposure to LF EMFs (16).

The aim of our study was to determine the effect of extremely low EMF frequencies on growth of yeasts of the genus *Saccharomyces cerevisiae*. The reason for the research is the desire to understand how it is possible to influence biological structures through an external LF EMF. As there are studies (17) on the presence of endogenous LF EMF around the range 0.4- 2 kHz in living organisms, the question of the possible influence of these frequencies on biological processes is relevant.

MATERIAL AND METHODS

The exposure system consisted of an incubator, an exposure coil, a shielding box, a signal generator, and an input harmonic signal amplifier. The main task of the incubator was to maintain the temperature of the samples, as it was a paired experiment. The incubator made of polystyrene and a polyethylene cover contained two polyethylene cavities inside for sample insertion. In addition to the built-in temperature measurement system, the incubator provided air circulation throughout the system. The exposure coil consisted of a PVC tube on which an enamelled copper conductor was wound in two layers of 100 turns. The geometric dimensions of the coil were: height 150 mm, width 167 mm, and a diameter of the wound conductor 0.9 mm. The yeast was exposed at a rms value of electric current I = 2 A (time course of sine shape) to the magnetic field with magnetic flux density B = 2.3 mT and frequency f = 900 Hz, which appeared as an appropriate maximum in the experiments (18). A signal generator (RIGOL DG 4162, Micronix) and a linear amplifier (Hubert A1110-05, Dr. Hubert GmbH) were used to condition the exposure signal. A multimeter was connected for the whole duration of the experiment and we continuously verify the value of the supply current. The control samples were placed in a metal shielding box. The measured magnetic flux density inside the shielding box was six-fold lower comparing to the center of the coil. The aim of shielding was to show in the results the difference between the natural process of multiplication and the growth of yeasts as opposed to the

effect of EMF. The same conditions (temperature, air supply) were ensured for the control sample as for the exposed one. The temperature inside the incubator was stabilised to 28°C during the whole experiment. The temperature was measured in both cavities of the incubator using NTC thermistors. The time of exposure to the EMF was different for individual time points. At 0 hours, paired samples were not exposed. At 2 hours, the exposed (E) sample was exposed for 2 hours and the control (C) sample was shaded for the same exposure time. The samples were then placed back in the incubator and exposed at additional time intervals (4 h, 6 h, and 8 h).

The material used in this study were yeast cells *Saccharomyces cerevisiae*. They were maintained at a constant temperature of 25°C in the laboratory under sterile conditions to achieve the optimal state for propagation and to prevent contamination. The yeast cells were obtained from the Science Park of the University of Zilina under the supervision of the Department of Electrical Engineering and Biomedical Engineering.

In the experiment we used YPD (Yeast Extract-Peptone-D-Glucose) medium which contained distilled water (95 % (w/v), peptone 2% (w/v), glucose 2% (w/v), and yeast extract 1% (w/v) (19). The preparation of the medium was followed by a pre-cultivation process lasting 16 hours. We inoculated a small sample containing the yeast (kept refrigerated at 4°C) into a flask with 10 ml of prepared YPD medium. The yeast medium was placed on a shaker. They were cultured for 16 hours at a speed of 180 rpm. Pre-cultured yeast solution with a volume of 1 ml was pipetted into two prepared flasks containing 30 ml of medium in each one. A paired experiment was performed with the same initial cell concentration in both samples (time 0 hours). The samples were placed into a dual cavity incubator (Fig. 1). One cavity of the incubator contained a radiation coil and the other cavity a shielding box. The radiation coil is a source of LF magnetic field and achieves approximately 90% of homogeneity. The flasks were closed with cotton plugs and an air supply was provided.

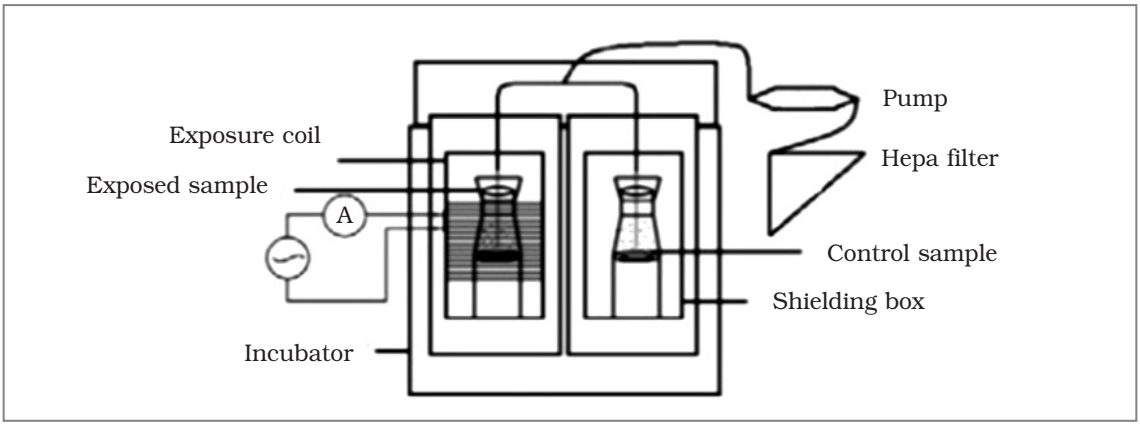

Fig. 1 Schematic representation of the measuring system (20).

The evaluation of growth dynamics was performed by the method of measuring cell concentrations during individual measurements. We observed cell growth (propagation) every two hours after sampling from the C and E solutions. Using a microscope (Primovert, Carl Zeiss Microscopy, LLC) we photographed the current number of yeast (Fig. 2). The yeast concentration *c* was calculated as the ratio of the number of yeast cells *n* located on one segment (one square in the microscope image) and the volume of the Neubauer chamber segment, the value of which is known as VS = $25x10^{-8}$ ml. In each measurement the number of cells was counted in one square (segment). The arithmetic mean of the number of cells was determined by adding the segments and divided by the number of measurements.

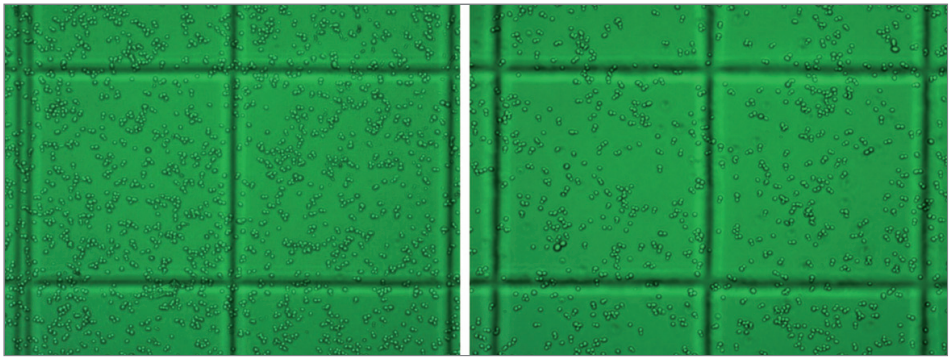

Fig. 2 C and E samples after 8 hours of EMF exposure at frequency f = 900 Hz

The values of concentrations passed the test of normality and the Paired test was used. Statistical significance of differences observed among the mean values was determined using Statistical Package for the Social Sciences (SPSS), $P < .05$ was considered significant.

RESULTS

The data obtained for C and E samples for selected time points are summarized in Table 1 and Table 2. According to Table 1 the values point to the fact that at analyzed frequency the C sample always acquired higher values. This means that the yeast multiplied faster under the maintained conditions in the laboratory (constant temperature, sterile environment) without an exposure to the EMF than in the case of the exposure (the E samples reached a smaller number of yeast cells). The values in Table 1 indicate that in the end

Table 1 Yeast cell concentrations [number of cells/ml]

f	Measurement number	Sample	0h	2h	4h	6h	8h
900 Hz	1.	C	$1.09x10^8$	$2.44x10^8$	$3.04x10^8$	$6.36x10^8$	$9.58x10^8$
		E	$1.09x10^8$	$2.14x10^8$	$2.09x10^8$	$5.96x10^8$	$8.50x10^8$
	2.	C	$1.24x10^8$	$2.27x10^8$	$3.37x10^8$	$7.23x10^8$	$1.59x10^9$
		E	$1.24x10^8$	$1.78x10^8$	$2.63x10^8$	$5.54x10^8$	$1.04x10^9$
	3.	C	$1.63x10^8$	$2.44x10^8$	$4.07x10^8$	$5.79x10^8$	$9.69x10^8$
		E	$1.63x10^8$	$2.12x10^8$	$3.03x10^8$	$4.68x10^8$	$7.42x10^8$
	4.	C	$2.20x10^8$	$2.72x10^8$	$3.97x10^8$	$7.62x10^8$	$1.87x10^9$
		E	$2.20x10^8$	$2.44x10^8$	$3.67x10^8$	$6.02x10^8$	$1.01x10^9$
	5.	C	$1.62x10^8$	$2.26x10^8$	$3.30x10^8$	$4.29x10^8$	$7.82x10^8$
		E	$1.62x10^8$	$1.82x10^8$	$2.72x10^8$	$3.20x10^8$	$5.74x10^8$

Abbreviations: E exposed sample, C control sample, f frequency.

of the measurement the concentration of cells in the C sample increases significantly compared to the E one. A slight inhibition persisted between 2 and 6 hours but the difference is especially notable between 6 and 8 hours (Figure 3).

In each of the measurements it was confirmed that the cell concentration in the E sample was always lower than in the C due to the LF EMF (Fig. 4). The mean C and E values, as well as concentration ratios of the samples at the selected time point from all performed measurements, are in Table 2. The arithmetic mean of the ratios of the concentrations of E and C cells in the end of the experiments for f = 900 Hz is 0.716 ± 0.13 ($p \leq 0.0482$).

Table 2 The mean values of cell concentration and concentration ratios from all measurements for selected time points. Paired test

Exposure	Exposure time	Control sample (Mean ± SD)	Exposed sample (Mean ± SD)	*P* Value	E/C concentrationratios (Mean ± SD)
900 Hz	0 hours	1.56 x10^8 ± 0.43	1.56 x10^8 ± 0.43	N/A	1 ± 0.00
	2 hours	2.43x10^8 ± 0.19	2.06x10^8 ± 0.27	0.0009*	0.85 ± 0.05
	4 hours	3.55x10^8 ± 0.45	2.83x10^8 ± 0.58	0.0055*	0.79 ± 0.09
	6 hours	6.26x10^8 ± 1.31	5.08x10^8 ± 1.18	0.0069*	0.81 ± 0.08
	8 hours	12.34x10^8 ± 4.70	8.43x10^8 ± 1.93	0.0480*	0.72 ± 0.13

Abbreviations: *Statistically significant difference.

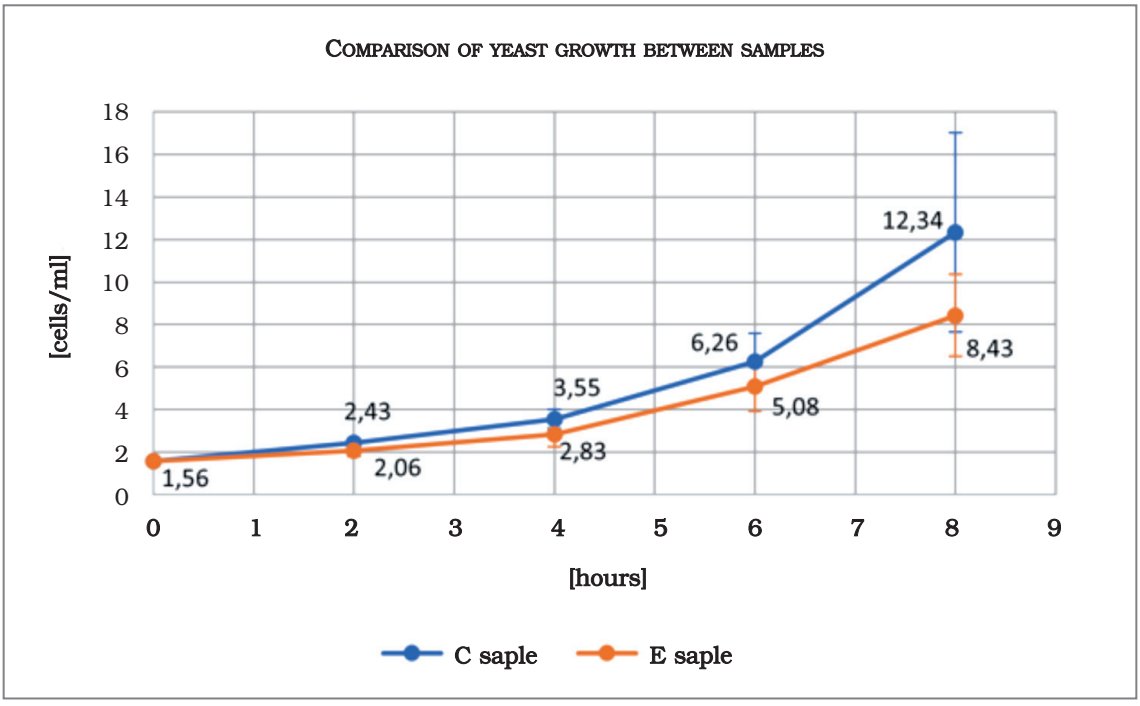

Fig. 3 Comparison of yeast growth at a frequency of 900 Hz

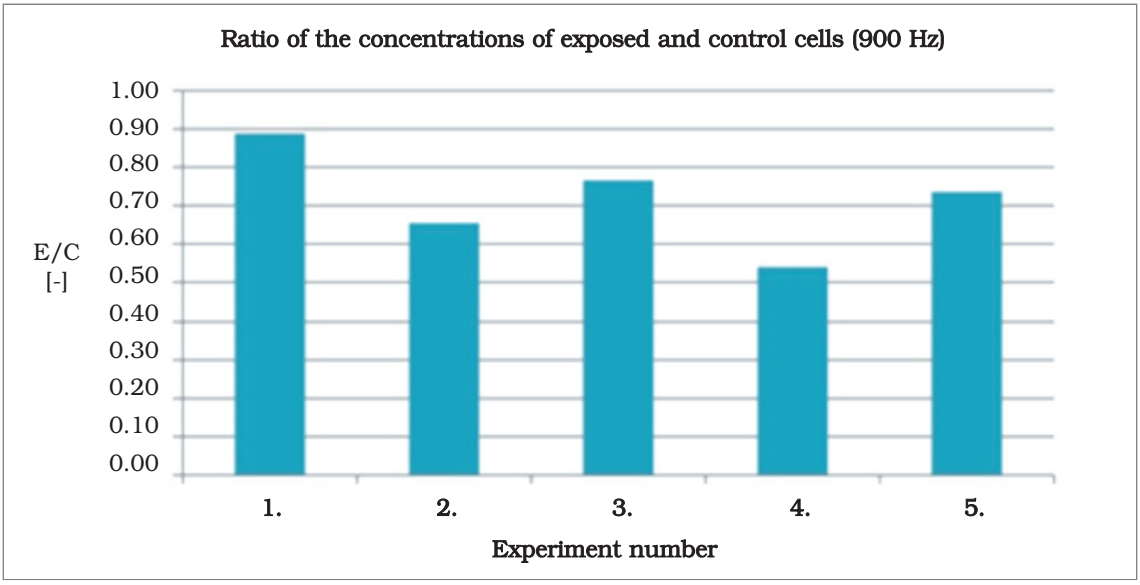

Fig. 4 The ratio of the concentrations of E and C samples after 8 hours

DISCUSSION

The results of this study suggest that the LF EMF at the given parameters of the experiment has an inhibitory effect on the growth of yeast cells. With increasing exposure time and parallel control culture an increase in the variance of cell numbers can be observed, which is characterized by a standard deviation. E/C concentration ratios reach a value lower than 1.0, which also confirms the previous assumption of the inhibitory effect. The highest decrease in yeast cell growth in the E sample compared to the C sample was found at a frequency of 900 Hz between 6 and 8 hours (31.69%). This was followed by a decrease of 20.28% between 2 and 4 hours, a decrease of 18.85% between 4 and 6 hours, and the smallest difference between the samples was 0 – 2 hours (15.23%). An interesting fact is that the SD increased significantly over time, which is actually seen in the decreasing P value. Several other studies have shown a frequency- dependent response of yeast. The effect of EMF on *Saccharomyces cerevisiae* was studied by Barabas et al. (2015), where an inhibitory effect of EMF on yeast cell growth was also observed at similar exposure parameters employing another method for evaluation of growth dynamics (18). Also, based on the study of Bereta et al. (20), which used the same evaluation method - Neubauer counting chamber, but was targeted to a different EMF frequency (1600 Hz), slower growth of yeast was confirmed. Our study combined the findings of both researches and confirmed the hypothesis that LF EMF has the ability to affect biological structures in the process of growth and reproduction. The results of the study were obtained at a frequency of 900 Hz, this allows further discussion of the possible action in the LF bands of EMF and their subsequent comparison or evaluation of which frequency achieves the highest inhibition, which may be the aim of another study. Also, further experiments could be aimed to observe biological effects at other parameters of magnetic flux density.

Although the effects of LF EMF on the growth of yeast is not a purely medical topic, the impact of this field has significantly affected human health in recent years. The human body is constantly exposed to subthreshold values of non-ionizing RF radiation. With the development of 5G networks comes the installation of antenna systems that will contain thousands of mini- antennas multiplying radiation. The EHS disease gives an area

of deeper investigation into the negative effects of EM radiation on some individuals. A study by Míšek et al. (2018) showed e.g. that students exposed to a short-term exposure of the head to RF EMF had significantly increased parasympathetic nerve activity compared to the control group when passing the ortho-clinostatic test affected by ANS in the lying position (21). Recent findings in the field of microbiology point to the fact that bacterial strains also respond differently to EMFs (modified bacterial growth or antibiotic resistance). Taheri et al. (2017) claim that an exposure of bacteria to a Wi-Fi router and RF simulator can cause the resistance of microorganisms to antibiotics. There has also been an inhibitory effect on the growth of *L. monocytogenes* and *E. coli*, which may have a significant effect on the management of infectious diseases (22). A different response to the action of EMF on microorganisms was also noted by Movahedi et al. (2019), where a mobile simulator emitted waves of 900 MHz frequency and indicated an increase in bacterial resistance in *S. aureus* and *P. aeruginosa* (23). The study by Crabtree et al. (2017) discusses the hypothesis that RF EMF from mobile phone can disrupt the microbiota of human skin (24). In summary, the action of EM radiation interferes with the biological processes of the microbiota and may result in pathological changes of the human body. Technological progress is unstoppable, however and only intensive research in this field may protect human health.

CONCLUSIONS

In our study, yeast cells of the genus *Saccharomyces cerevisiae* were exposed to LF EMF with a frequency of 900 Hz. A significant inhibitory effect of EMF (magnetic flux density 2.3 mT, rms value of electric current 2 A, frequency 900 Hz, EMF exposure time 0 h, 2 h, 4 h, 6 h, and 8 h) on the reproduction and growth of unicellular organisms was observed by using a comparative method. The results indicated that at given values the LF EMF has inhibitory effects on the life cycle of the yeast cell. Thus, it is appropriate to extend the study to include additional exposure tests with other frequencies.

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DETECTION OF SUBCLINICAL PAROXYSMAL ATRIAL FIBRILLATION AND ITS CORRELATION WITH CANDIDATE GENES IN PATIENTS WITH CRYPTOGENIC ISCHEMIC STROKE AND TIA

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Abstract

Introduction: Cardioembolic etiology is assumed to be the most frequent cause of cryptogenic strokes. The detection of subclinical paroxysmal atrial fibrillation (AF) is important in the correct choice of preventive treatment. The aim of this prospective study was to detect the incidence of AF in patients with a cryptogenic stroke or transient ischemic attack (TIA) and to evaluate the association between the presence of AF and selected single-nucleotide polymorphisms (SNP).

Methods: Patients with a cryptogenic stroke/ TIA (n=100) and a control group (n=15) of volunteers without significant cardiovascular disease were included in the study during the period of 2014 to 2019. To detect AF they underwent 12 months of ECG monitoring using an implanted loop recorder (ILR). Genotyping for SNPs rs10033464, rs2200733, rs225132, and rs2106261 was performed by a high resolution melting analysis.

Results: We found AF to be present in 24 (24%) patients with a cryptogenic stroke/TIA, versus no subjects in the control group. The SNPs rs2106261, rs2200733, rs225132, and rs10033464 were not found to be associated with AF in our study (p=0.240; 1.000; 0.887; 0.589). However, a weak trend for a higher frequency of rs2106261 risk allele A homozygotes was observed in the patients with AF compared to the patients without AF (0.416 vs. 0.263, p=0.073). Homozygotes for allele A of *rs2106261* were also present in a significantly higher frequency in AF patients compared to the controls (0.416 vs. 0.133, p = 0.012).

Conclusion: In our study paroxysmal AF was a probable etiological factor in 24% of patients with cryptogenic ischemic stroke / TIA during the 12 months of monitoring. The homozygous allele A of rs2106261 was identified to be the possible genetic risk factor of AF, but this should be verified in larger cohorts.

The study has been registered at www.clinicaltrials.gov, identifier NCT02216370.

Key-words: transient ischemic attack, cryptogenic stroke, atrial fibrillation, implantable loop recorder, single-nucleotide polymorphisms

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INTRODUCTION

An ischemic stroke (IS) is a heterogeneous multi-factor polygenic disease with a high mortality rate and long-term consequences. (1) One of the known, important, and independent risk factors for IS is atrial fibrillation (AF). (2) Its early detection is of fundamental prognostic importance. Paroxysmal AF is often considered a probable cause of cryptogenic stroke. (3) The detection of subclinical paroxysmal AF in this case is difficult because it can be asymptomatic, mainly in older people, and traditional 12-lead or Holter ECGs have a low level of detection due to the limited length of the recording. In recent years the higher detection of AF is due to the introduction of long-term monitoring strategies. Non-stop long-term ECG monitoring using implantable devices is a better and clinically more effective technique than conventional monitoring, in particular following a cryptogenic stroke. In order to achieve maximum AF detection further factors should be identified in order to choose patients for a long-term monitoring. Greater attention is paid to genetic studies under the assumption that genetic tests might help define the subtype of a cryptogenic stroke. (4–8)

The aim of the work was to define the following in a group of patients with a cryptogenic stroke/ transient ischemic attack (TIA): 1) the presence of AF using an implantable ECG loop recorder (ILR); 2) the number of recurrences of stroke/ TIA during the following 12 months; 3) the presence of new ischemic lesions during a magnetic resonance (MRI) examination 12 months later; 4) whether there is a relation between newly-diagnosed AF in our group of Slovak patients and the find of selected single-nucleotide polymorphisms (SNPs) in literature associated with AF and cardioembolic infarction.

METHODS

Characteristics of the groups

One hundred patients with an acute ischemic stroke or TIA were included in the study. The patients were hospitalised within 72 hours from the onset of the ischemic stroke at the Clinic of Neurology of the Faculty Hospital in Nitra from 2014 to 2019 and they met the definition of cryptogenic etiology according to TOAST criteria (inclusion and exclusion criteria) during the screening period. Transient ischemic attack was defined as a temporary neurological deficit of vascular origin without acute ischemic MRI changes. The possible etiological factors of ischemic stroke were excluded in all patients by: CT and MR imaging of the brain and intra/extracranial arteries, hematological testing for thrombophilia, rheumatological tests for vasculitis, 14 days lasting 12-lead Holter ECG for detection of paroxysmal AF, transthoracic and transoesophageal echocardiography for cardioembolism. The control group was made up of 15 volunteers without a significant cardiovascular and cerebrovascular disease adjusted by age and gender. The patients were monitored for 12 months. All patients and volunteers in the control group signed an informed consent form.

Examinations

The diagnosis of an ischemic stroke required consistent neuroimaging findings on CT and MRI. In control individuals MRI had not been performed before participating in the study. All subjects in patient and control groups in addition underwent a follow-up MRI once the monitoring was finished. Standard non-contrast CT and CT angiography of the aortic arch, cervical and intracranial arteries (SIEMENS Somatom Definition, dual source 2x128, Stellar upgrade, SIEMENS Somatom Edge 128), and brain MRI (SIEMENS Skyra 3.0T, SIEMENS Magnetom Avanto 1.5T, SIEMENS Magnetom Symphony 1.5T (protocol T2wTSE SAG + TRA, FLAIR T2wTIRM dark fluid - TRA, DWI – epi2ddiffusion – TRA + ADC, MRA-TOF3D multislab – TRA + MPR + MIP + VRT, CE imag.-i.v. inject. application + aqua flush 30ml – T1w mpr ISO – TRA + MPR / COR + SAG) were carried out at the JESSENIUS diagnostics centre. The CT and MRI findings were evaluated by independent radiologists.

Implantation of a long-term ECG loop recorder

The implantation of a ILR (REVEAL LINQ / XT, Medtronic Inc., Minneapolis, USA) was carried out within 6 months of the onset of the ischemic stroke/ TIA as arranged with a cardiologist in the following sites: Clinic of Cardiology of the Faculty Hospital in Nitra; Kardiocentrum Nitra s.r.o.; Department of Arrhythmology of the National Institute of Cardiovascular Diseases and the Clinic of Internal Medicine of the Faculty Hospital in Nové Zámky. AF was defined as an episode of irregular cardiac rhythm without the presence of a P wave, lasting for more than 2 minutes. The ECG output obtained from the ILR during check-ups was assessed by an arrhythmologist.

Check-up examination

During the final check-up examination after 12 months we assessed the AF finding, the result of the follow-up brain MRI examination, the recurrence of acute stroke, and a change in treatment in the secondary prevention of acute stroke. In cases where AF was detected by ILR in the course of the 12-month monitoring period the patients and the control subjects underwent an unplanned neurological check-up with a change from antiplatelet treatment to anticoagulation. An unplanned neurological check-up was also undergone in the case of a recurrence of acute stroke.

Genetical analysis

The genetic examination was carried out at the Department of Molecular Biology and Genomics, the Jessenius Faculty of Medicine in Martin and BioMed, Comenius University in Bratislava, in cooperation with the Department of Clinical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, and University Hospital Martin. Four SNPs were selected for genetic examination: *rs10033464* (*PITX2c gene*), *rs 2200733* (*PITX2c gene*), *rs225132* (*ERRF11 gene*), and *rs2106261* (*ZFHX3 gene*). The characteristics of the analysed SNPs are shown in Table 1.

Table 1 Selected single-nucleotide polymorphisms (SNP)

SNP	*Genetic locus*	*Chromosome*	*Major / risk allele*	*Susceptibility, population*
rs2106261	ZFHX3	16q22	G / A	AF, Europeans ≈11% Han Chinese
rs2200733	PITX2c	4q25	C / T	AF, Europeans
rs225132	ERRF11	1	T / G	All types of ischemic stroke, coronary heart disease, Europeans
rs10033464	PITX2c	4q25	G / T	AF, Europeans

Abbreviations: AF – atrial fibrillation

The samples of peripheral blood were taken and dispensed into 3 ml tubes containing 5,4 mg of EDTA (Vacutest Kima S.r.l., Italy). DNA was isolated using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). The concentration and purity of the DNA were determined using Nanodrop (ND-1000, NanoDrop Technologies, Wilmington, DE). All samples were diluted in sterile distilled water to a 25-50 ng/µl final concentration. PCR amplification and genotyping was performed by a High Resolution Melting (HRM) analysis using the Roche LightCycler® 480 Instrument (Roche, USA). The reaction volume consisted of 10 µl of LC480 HRM Master Mix, 2,4 µl of $MgCl_2$ (25 mM), 2 µl of genomic DNA sample (25–50 ng),

and 0.4 µl of 10µM primers (Ecoli, Czech Republic) filled up to a total volume of 20 µl by redistilled water. The oligonucleotide primers were designed with the online uDesignSM application (www.dna.utah.edu/udesign/index.html). The primer sequences are shown in Table 2. The reaction conditions for rs10033464 and rs2106261 were: 95°C for 10 minutes, (95°C for 10 seconds, 66°C for 15 seconds, 72°C for 10 seconds) x 45 cycles; 72°C for 5 minutes. Heteroduplex PCR was performed at 95°C for 1 minute, 40°C for 1 minute; and c) HRM: 65 to 95°C with a ramp rate set to 0.02°C/second and 25 acquisitions/°C. The reaction conditions for rs225132 and rs 2200733 were: 95°C for 10 minutes, (95°C for 10 seconds, 60°C for 15 seconds, 72°C for 10 seconds) x 45 cycles; 72°C for 5 minutes. Heteroduplex PCR was performed at 95°C for 1 minute, 40°C for 1 minute. HRM analysis was performed at 65 to 95°C with a ramp rate set to 0.02°C/second and 25 acquisitions/°C. To verify the quality of genotype determination several samples from each SNP were bi-directionally sequenced by Sanger sequencing. PCR fragments were purified with ExoSap-IT (Applied Biosystems, Foster City, CA, USA). Sequencing of SNPs was performed using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Ca, USA) on the ABI PRISM 3130x1 automatic sequencer (Applied Biosystems, CA, USA). Sequence data analysis was carried out by using Chromas software (http://www.technely-sium.com.au/chromas.html).

Table 2 Primers used for HRM

SNP	*Primer sequence*
rs2106261	F 5 CACAGATAGAGCTCGTCCAGAGAATTG
	R 5′ GCCACTTGGATATTTTAATGGATGGTT
rs2200733	F 5′ GTGGTACTTGGGTTTTGATTTTGA
	R 5′ ACTACCTTAAATATTACCTGTTCTAATTTTCTC
rs225132	F 5′ TCAGTGAAGAGGTGGTGAATGTAGGAAT
	R 5′ ACCATGTGCCATGAATGCAA
rs10033464	F 5′ CAATTTAAAATTTTCTTTTTTTACATTGTTAGAGTCAAGAA
	R 5′ CTCAGAGCTTGATGAAAGCACT

Abbreviations: AF – atrial fibrillation

Statistics

We used common methods of descriptive statistics. We evaluated the association between AF and the other categorical variable, SNPs, using a contingency table. The zero hypothesis on the non-existence of an association between atrial fibrillation and another categorical variable (SNP) was tested using a Pearson's Chi-squared test with Yates' continuity correction and a Cochran-Armitage trend test. All the data analyses were done in R [15], ver. 3.5.2.

Registration of the study

The study has been registered at www.clinicaltrials.gov under the identifier NCT02216370.

RESULTS

Demographic data

The mean age of the subjects in the patient group was 70.1 years (28–87) vs. 75.4 years (35–90) in the control group. The ratio of women in the patient group was 30% (30) vs. 66.6% (10) in the control group. 90% (90) of patients had experienced a cryptogenic ischemic stroke with a moderate neurological deficit; the median NIHSS was 6.9 (0–15). The ratio of TIA in the patient group was 10% (10). The subjects in the patient group showed an average CHA2DS2-VASc score of 3 (2–5) and 1.4 (0–3) in the control group. A total of 68 (68%) of the subjects in the patient group had an arterial hypertension in their personal history (18 (75%) patients with detected AF vs. 9 (60%) of the volunteers in the control group). In the patient group a total of 14 (14%) patients had diabetes mellitus, of which 1 (4.2%) patient had detected AF vs. 2 (13.3%) volunteers in the control group. Clinical characteristics of the involved individuals are summarised in Table 3.

Table 3 Clinical characteristics of involved individuals

Characteristics	*With AF (n=24)*	*Without AF (n=76)*	*Controls (n=15)*
Age (median)	64.29	62.59	75.46
Women % (n)	29.16 (7)	43.42 (23)	66.6 (10)
Cryptogenic stroke	22	68	0
TIA	2	8	0
NIHSS median (range)	6.6 (0–13)	7.1 (0–15)	0
CHA_2DS_2VASc median (range)	3 (2–4)	3 (2–5)	1.4 (0–3)
Hypertension % (n)	75 (18)	65.8 (50)	60 (9)
Diabetes mellitus % (n)	4.17 (1)	17.11 (13)	13.3 (2)

Abbreviations: TIA – Transient ischemic attack, AF – Atrial fibrillation, NIHSS – The *National Institutes of Health Stroke Scale*, n – number

Detection of atrial fibrillation, recurrence of acute ischemic stroke, and change of treatment

AF was confirmed in 24% (n = 24) of the patients out of 100 in the patient group. The mean time interval between ILR and AF was 3.9 months (1–11 months). AF was not confirmed in any volunteer in the control group. Within the course of the 12-month monitoring period no subject in the patient nor control groups experienced a clinical or asymptomatic recurrence of an ischemic stroke or a systemic embolism. After the 12 months of monitoring MRI did not reveal any new ischemic brain lesions in the patient and control groups. In the patient group with AF (n = 24) the antiplatelet therapy was changed immediately to anti-coagulants (Table 4).

Table 4 Detection of atrial fibrillation, change in MRI finding, change in preventive treatment after 12 months

Characteristics	*Patient group (n = 100)*		*Control group (n = 15)*
	With AF (n=24)	*Without AF (n=76)*	*Without AF (n=15)*
Implantation of ICM% (n)	100 (24)	100 (76)	100 (15)
Time before the implantation of ICM (m)	2.5 (1–4)	1–6	1–6
Time preceding detection of AF (m)	3.9 (1–11)	0	0
Recurrence of IS /SE	0	0	0
New MRI ischemic lesions after 12 m % (n)	0 (0)	0 (0)	0 (0)
Change in preventive treatment % (n)	100 (24)	0 (0)	0 (0)

Abbreviations: IS = ischemic stroke, SE = systemic embolisation

3. Analysis of the association of selected SNPs with AF in the patient group

The genotype frequencies of the analysed SNPs in the subgroups of patients and in the controls are shown in Table 5.

SNP *rs2106261* had no statistically significant association with AF (p = 0.240). However, a weak trend for a higher frequency of homozygous form of rs2106261 risk allele A in patients with AF compared to the patients without AF was observed (41.6% vs. 26.3%, p = 0.073). Allele G of *rs2106261* was present in 14 (58.4%) patients with AF vs. 56 (73.7%) without AF. The trend for association of *rs2106261* with AF was also found when compared the patients with AF to the controls (p = 0.058). The AA homozygotes were 10 patients with AF compared to 2 individuals in the control group and the difference was statistically significant (41.6% vs. 13.3%; p = 0.012). Allele G of *rs2106261* was found in 14 (58.4%) patients with AF vs. 13 (86.7%) volunteers in the control group.

We found a weak trend for the association of rs2106261 with AF (p = 0.076). Homozygotes for allele A of *rs2106261* were present in a significantly higher frequency in AF patients compared to the controls (41.6% vs. 13.3%, p = 0.012).

A positive finding of rs2200733 risk allele T homozygotes was observed in 5 (21%) patients with AF vs. 15 (20%) patients without AF. Allele C of *rs2200733* was found in 19 (79%) patients with AF vs. 61 (80%) patients without AF. A statistically significant association of *rs2200733* with AF was not found (p = 1). When comparing patients with AF to the control group, homozygotes for risk allele T of *rs2200733* were in 5 (20.8%) patients vs. 3 (20%) volunteers in the control group. Allele T of *rs2200733* in homozygous form was present in 19 (79.8%) patients with AF vs. 12 (80%) volunteers in the control group. In patients with AF compared to the control group, *rs2200733* did not show any statistically significant association with AF (p = 0.945).

Homozygotes of rs225132 minor allele G were 5 (20.8%) patients with AF vs. 19 (25%) patients without AF. Ancestral allele T of *rs225132* was found in 19 (79.2%) patients with AF vs. 57 (75%) patients without AF. A statistically significant association of *rs225132* with AF was not confirmed (p=0.887). When comparing patients with AF to the control group, homozygous allele G of *rs225132* was found in 5 (20.8%) patients vs. 5 (33.3%) volunteers in the control group. Allele T of *rs225132* was confirmed in 19 (79.2%) patients with AF

Table 5 Genotype frequencies of the analysed SNPs in patients and controls

	SNP	*rs2106261*		*rs2200733*		*rs225132*		*rs10033464*	
		AA	*GG + GA*	*TT*	*CC + CT*	*GG*	*TT + TG*	*TT*	*GG + GT*
Patients (n = 100)	*without AF (n = 76)*	0.263 (n = 20)	0.737 (n = 56)	0.200 (n = 15)	0.800 (n = 61)	0.250 (n = 19)	0.750 (n = 57)	0.105 (n = 8)	0.895 (n = 68)
	with AF (n = 24)	0.416 (n = 10)	0.583 (n = 14)	0.208 (n = 5)	0.792 (n = 19)	0.208 (n = 5)	0.792 (n = 19)	0.042 (n = 1)	0.958 (n = 23)
$^{2\ test}$ p-value (with vs. without AF)		0.240		1.000		0.887		0.589	
Controls (n = 15)		0.133 (n = 2)	0.867 (n = 13)	0.200 (n = 3)	0.800 (n = 12)	0.333 (n = 5)	0.667 (n = 10)	0.267 (n = 4)	0.733 (n = 11)
$^{2\ test}$ p-value (AF patients vs. controls)		0.058		0.945		0.884		0.261	

vs. 10 (66.4%) healthy volunteers. A statistically significant association of *rs225132* with AF was also not shown when patients with AF were compared to the controls (p=0.884).

Homozygous risk allele T of rs10033464 was present in 1 (4.2%) patient with AF vs. 8 (10.5%) patients without AF. Allele G of *rs10033464* was found in 23 (95.8%) patients with AF vs. 68 (89.4%) patients without AF. A statistically significant association of rs10033464 with AF was not confirmed (p=0.589). When comparing patients with AF to the control group, homozygous *rs10033464* risk allele T was present in 1 (4.2%) patient vs. 4 (26.7%) volunteers in the control group, and the difference was statistically significant (p=0.047). Ancestral allele G of *rs10033464* was found in 23 (95.8%) patients vs. 11 (73.3%) volunteers in the control group.

DISCUSSION

In our study we found that paroxysmal AF was a probable etiological factor in 24% of patients with cryptogenic ischemic stroke/ TIA. In the control individuals paroxysmal AF was not present during the 12 months of monitoring. In the randomised control CRYSTAL-AF clinical study the AF detection after 12 months was lower compared to our findings, 12.24% versus 24%. In the above-mentioned study a 30.0% detection rate was only achieved after 36 months of monitoring. (9) In the same manner, a lower detection rate of AF over an average monitoring period of 365 days, compared to our results, was found by Marks et al. (2019) in a retrospective study based on real practice. Using an ILR the authors detected a subclinical AF in 35 patients (19.6%) in a group of 178 patients with cryptogenic ischemic stroke. (10) The detection rate of AF in our patients approximately correlates with the data of the meta-analysis of 28 studies processed by Tsivgoulis et al. (2019) in patients with cryptogenic ischemic stroke and embolic stroke of underdetermined source (ESUS) (of which 10 studies monitored patients from 6 to 12 months). (11) The cumulative AF detection rate in patients with ILR was 26% of the above-mentioned meta-analysis. Depending on the duration of monitoring, the authors found significant differences in the AF detection rates ($p < 0.001$) (<6 months: 5%; ≥6 and ≤12 months: 21%; >12 and <24 months: 26% > 24 months: 34%). They did not confirm any association with other patient characteristics, such as the subtype of ischemic stroke (cryptogenic vs. ESUS), or the time from the beginning of the ischemic stroke to the implantation of the ILR. These findings can be extrapolated to our research. The time of ILR implantation in all our patients within 6 months of the ischemic stroke/ TIA, which was needed for the organisation, performance of examinations and assessment, did not affect the AF detection rate.

In all 24 patients (100%) with AF an antiplatelet therapy was changed to anticoagulation following the recommendations of the ESC (European Society of Cardiology, 2016). (12) We noted no recurrence of ischemic stroke either in the patients or in the control group over the monitored period (12 months), probably due to a short period and an appropriate change of treatment. Unlike our findings, a population study in Oxfordshire, United Kingdom, performed by Li et al. (2015) in patients with a first TIA or cryptogenic ischemic stroke revealed that a 10-year risk of death was 46%, vascular death was 15%, and the recurrence of any form of ischemic stroke was 32%. The risk of recurrence of ischemic stroke/ TIA in this study was lower compared to patients with a cardioembolic etiology ($p = 0.003$). (13) We can only speculate whether the individual efficacy of antiplatelet therapy, possibly depending on p-selectin expression, could be related to a recurrency of ischemic stroke/TIA (14).

A follow-up brain MRI after the 12 months of monitoring did not reveal new asymptomatic ischemic lesions in the patient and control groups. Studies dealing with the MRI detection of new ischemic lesions after a cryptogenic ischemic stroke focus more on proof of low-level symptomatic carotid stenosis, atherosclerosis of small intracranial arteries, or foramen ovale patens as etiological causes. On the other hand, Bal et al. (2012) in a group of 333

(48%) patients with cryptogenic ischemic stroke, out of the total number of patients with a mild stroke/ TIA (National Institutes of Health Stroke Scale score NIHSS ≤3), found that 6.6% of patients had new lesions at the 30-day and 14.5% of patients at the 90-day MRI follow-up. (15) This population showed a high rate of silent radiological recurrence, which points to the disease activity. However, the study does not mention the type of therapy administered as part of the secondary prevention of ischemic stroke. In our study the patients had a higher NIHSS score 7, they were given an antiplatelet therapy and an anticoagulation therapy after the finding of AF. The relation between AF and brain lesions caused by a silent cerebral infarction (SCI) at an undefined time, diagnosed by MRI, has been examined in several studies. For example, the Framingham Offspring Study (Das et al., 2008) found a higher risk of SCI in patients with AF (OR 2.16). (16) On the contrary, Gaita et al. (2013) found white matter lesions in 92% of patients with persistent AF, in 89% of patients with paroxysmal AF, and in 46% of patients without AF. A relation between SCI prevalence and the duration of AF was not found. (17)

Regarding the genetic analysis, in the case of rs2106261, we did not confirm a statistically significant association with AF (p=0.240). However, we found a slight significant trend towards a higher frequency of *rs2106261* AA homozygotes in patients with AF compared to the control individuals (p=0.076). Interestingly, a significantly higher frequency of AA homozygotes was also present in patients with AF when compared to the controls (p=0.012). Our results can be interpreted in accordance with the published data. Benjamin et al. (2009) in the meta-analysis of GWAS in patients with AF identified of a new susceptibility locus *ZFHX3*, *rs2106261* (RR = 1.19; $p = 2.3 \times 10^{-7}$). (4) These findings were replicated in a cohort study of Psaty et al. (2009) from the German AF Network (OR=1.44; $p = 1.6 \times 10^{-11}$; combined RR = 1.25; combined $p = 1.8 \times 10^{-15}$). (5) It is interesting to note that the majority of available publications that identified the association of this SNP variant with AF mainly studied in Asian population by Li et al. (2011) and Zaw et al. (2017). (18, 19) In our study the rs2200733, rs225132, and rs10033464 did not show any significant association with AF. Our findings contradict Henninger et al. (2016) who found that patients with a cardioembolic stroke have a high-risk genetic profile, even despite age and numerous clinical co-morbidities. (20) Pulit et al. (2018) found that a polygenic risk score of AF explains ~20% of the hereditary component of the cardioembolic risk of ischemic stroke. (21) Contrary to our findings, Gretarsdottir et al. (2008) and Gudbjartsson et al. (2009) consider the variant rs2200733 as one of the most important genetic polymorphisms associated with AF and cardioembolic stroke. (6,7) This finding has been consistently replicated and validated in several cohorts of patients with a statistical significance of $p<0.05$, in European and Asian populations by Wnuk et al. (2011), Viviani et al. (2008), Cao et al. (2013), and Sun et al. (2016). (22-25) The published information concerning *rs10033464* is inconsistent and our negative findings indirectly and partially match the data in literature. Lemmens et al. (2010) found that *rs10033464* is only weakly associated with AF and this variant has demonstrated no relation to ischemic stroke. (26) However, in an isolated population of patients in Iceland, Gretarsdottir et al. (2008) reported a strong association between *rs10033464* risk allele T and cardioembolic stroke (OR = 1.27; $p = 6.1 \times 10^{-4}$). (6) A strong association between *rs10033464* and AF was also confirmed by Kääb et al. (2009) in cohorts of patients of European origin: the Framingham Heart Study (327 AF patients and 2006 controls, OR = 1.34; 95% CI 1.03–1.75; p = 0.031), the German AF Network (1715 patients with AF and 4073 controls, OR = 1.30; 95% CI 1.13-1.51; p =0.0002), and Rotterdam Study (910 AF patients and 5496 controls, OR = 1.17; 95% CI 1.13-1.51; 0.99 – 1.38, p = 0.07). (8) On the other hand, the association of rs10033464 with AF was not found in Vanderbilt AF Registry study that was performed in 556 patients with AF and 598 controls without AF (OR = 1.16; 95% CI 0.86-1.56; p = 0.33). (8) Interestingly, the autosomal dominant loci in *ankyrin B protein 2* gene on chromosome 4q25 were showed to be associated also with ventricular arrythmias, particularly long QT syndrome. (27)

In the only publication dealing with *rs225132* in context of AF, Traylor et al. (2012) (METASTROKE Collaboration) identified an association between the rs225132 variant and AF in patients with ischemic strokes ($p=6.3\times10^{-8}$ a 5.9×10^{-8}), and this is not in concordance with our findings. (28)

CONCLUSION

In our study we identified the high sensitivity of long-term ECG monitoring using an ILR for detecting subclinical paroxysmal AF as a possible etiological factor of cryptogenic ischemic stroke/TIA comparable to the published sources. In the majority of our patients AF would probably not have been detected at all under a shorter ECG monitoring. The causal relationship between the detected AF and ischemic stroke will require a further analysis. A neurogenic type of AF known as AFDAS (atrial fibrillation detected after stroke), which is triggered by an acute ischemic brain lesion, could explain the time correlation between AF and stroke in some of our patients.

One limitation of the study could be the selection of patients using the TOAST classification that is currently being shown to be insufficient, in particular for defining subgroups of patients with suspected subclinical AF. The selection of patients currently requires a close multidisciplinary (neurology-radiology-cardiology) cooperation, the inclusion of new diagnostic procedures, markers, predictive risk scores that were not known at the time when the methodology for this study was being prepared, or were not routinely carried out. Due to a relatively small number of included patients and control subjects, defining a causal relation between paroxysmal AF detected after ischemic stroke (AFDAS) might appear problematic and will require a further sub-analysis.

For genetic testing we chose four well-known single nucleotide polymorphisms possibly associated with AF - rs10033464 in *PITX2c* gene, rs2200733 in *PITX2c* gene, rs225132 in *ERRF11* gene, and *rs2106261* in *ZFHX3* gene. None of these polymorphisms was shown to be significantly associated with AF in our study. However, in patients with cryptogenic ischemic stroke/ TIA a positive trend for the association of rs2106261 allele A homozygotes with AF was observed. This insignificant direct correlation may relate to the above-mentioned neurogenic subtype AFDAS which we did not analyse in our study. The possible confounding factor of these findings can be a relatively small cohort of examined individuals, which was due to the financial limitations of a study, especially by the number of available implantable ECG loop recorders. At present, however, 150 candidate genes have been identified for AF. Their variants could lead to a structural transformation of the heart in the form of atrial cardiomyopathy during intra-uterine development, or induced by stress in adulthood. For this reason, to confirm our findings and to clarify the exact mechanisms of involvement of candidate SNPs in AF and cryptogenic ischemic stroke, the future genetic and mainly functional studies including a whole-genome sequencing analysis in larger cohorts of individuals in different populations are necessary. We assume that including a genomic approach in the management of patients with cryptogenic ischemic stroke/ TIA might offer a narrower selection of patients for the implantation of a financially costly ILR and an earlier onset of targeted preventive treatment.

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DISTRIBUTION OF METASTASES IN ENT AREA – COMPARISON OF THEORY AND PRACTICE

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Abstract

In head and neck cancers the occurrence of nodal metastases is the most important prognostic factor. Their early diagnosis is crucial for proper treatment. Detection of early metastases is still very difficult. Predictive diagnostic methods such as the sentinel lymph node detection is limited by the occurrence of skip metastases. At our Clinic we prefer a selective neck dissection based on a surgical treatment of predilected lymphatic spread area for each type of head and neck tumor with a preservation of non-lymphatic structures of the neck. The main objective of this article is to analyze the distribution of neck metastases and to study the frequency of skip metastases in head and neck cancer.

Key words: skip metastasis, head and neck cancer, lymph node dissection

INTRODUCTION

Metastasis is a secondary malignant tumor which is formed by breaking away from the primary cancer and spreading through blood, lymph system, or by direct spread to the surroundings, and form new metastatic tumors in other parts of the body. The ability to metastasize is one of the essential signs of malignancy (1, 2).

One of the most important prognostic factor in head and neck cancers is the occurrence of nodal metastases (1). The incidence of neck metastases (MTS) reduces patients´ overall survival by almost 50% and increases the risk of disease recurrence (3). Only a smaller per cent of oncologic patients die as a result of a size of the primary tumor, the death is most often caused by metastases (2). Early diagnosis and identification of the exact location of MTS is crucial for choosing the right surgical treatment (1, 4).

Metastases in the neck are manifested mostly by solid, slow-growing swelling, the skin above is intact, without local inflammation. Initially, the nodes are movable towards the skin and the base, gradually occurs infiltration of the surrounding structures and fixation of the tumor. Nodal metastases are usually unilateral, bilateral occurrence is typical in malignancies of the tongue root and nasopharynx or indicates extension of the primary tumor to both sides or a generalization of the disease. Characteristic sign in carcinomas of the tonsils and the tongue root are cystic metastases in the jugulodigastric region. In advanced metastases we can observe necrotic and inflammatory changes in lymph nodes with infiltration of surrounding structures (1, 5, 6).

Other associated symptoms such as dysphagia, odynophagia, otalgia, dysphonia, or dyspnoea are determined by the primary tumor. General non-specific symptoms include increased fatigue, anorexia, and cachexia (2).

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Based on the location of the primary tumor we can predict the probable spread of metastatic disease to lymph nodes. According to the American Academy of Otolaryngology

Head and Neck Surgery classification, also known as Robbins classification, lymph nodes of the neck are divided into 6 areas (1, 3).

Tumors of the **nasopharynx** have a high incidence of metastases regardless of the stage of the disease. They occur in 70–80% of patients in areas II and V (1).

The **oropharyngeal** region is one with the largest lymphatic supply, therefore, at the time of diagnosis, up to 75% of patients have established nodal metastases.

In **hypopharyngeal** tumors metastases are a little less frequent – 60% (5, 6, 9).The most often affected are the ipsilateral deep jugular nodes in areas II, III, and IV. If the tumor extends beyond the midline, metastases may be bilateral – e.g. in tongue root or soft palate cancer (1, 5, 9).

Tumors of the **larynx** are one of the most common malignancies in head and neck, accounting for approximately 25–30% (4). In supraglottic and infraglottic tumors the incidence of MTS is 35–45%, similar to the oropharyngeal region, they have large lymphatic supply, so bilateral nodal involvement is not uncommon. In contrast, glottic carcinomas, due to a weak lymphatic supply, metastasize rarely, only in advanced stage of the disease. Laryngeal tumors typically metastasize to areas II, III, and less commonly to areas IV and VI. The spread of MTS in areas I and V is exceptional and does not occur without the simultaneous presence of MTS in areas II and III (1, 4, 5).

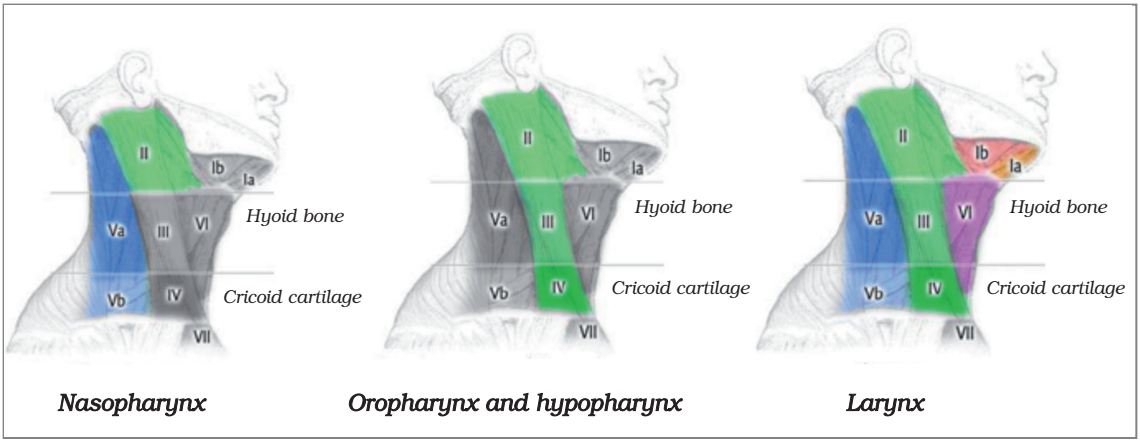

Fig. 1 Metastatic spread according to the location of primary tumor (10)

An early detection of nodal metastases remains a major challenge despite the constant development of imaging modalities (7). Commonly available screening methods help us to detect approximately 70–80% of metastases, while small metastatic nodes without necrosis and clinically obvious symptomatology remain undetectable in 10–70% of patients (3). Regarding this fact, radical, elective neck dissections have been performed in the past to detect occult metastases before their clinical manifestation (1). As a result, approximately 60–70% of patients were overtreated, as they underwent redundant, invasive procedures with significant associated complications (7).

Therefore, it was necessary to develop a predictive diagnostic method that can increase diagnostic accuracy, including negative predictive value, enough to reduce unnecessary elective dissections of the neck.

Sentinel lymph node (SLN) detection and biopsy was considered to be such a method. SLN is defined as the first node into which lymph enters from the primary tumor. The aim of the biopsy is to detect occult metastases - if the SLN is invaded by malignancy, the spread of

the disease to other descending nodes is highly probable and block neck dissection is indicated (8).

However, the limiting factor of this method is the occurrence of skip metastases. This term is used when a tumor cell skips the nearest descending lymph node and forms a metastasis in the next node.

The occurrence of skip metastases has been confirmed by several retrospective studies. Shikharani and colleagues reported a 6.7% incidence of skip metastases in a group of 30 patients with oral cancer (9).

In recent years, positron emission tomography (PET) has been increasingly used. It is a functional imaging technique that uses radiotracers to visualize and measure

metabolic changes in tissue. In detection of malignant lymph nodes, PET-CT has 90% sensitivity and 94% specificity (1). Currently, one of the leading methods is dual-source multienergy CT with texture analysis (7).

The aim of our work is to analyze the distribution of neck metastases and to study the frequency of skip MTS in head and neck cancer.

METHODS

At our Clinic we prefer a selective neck dissection based on a surgical treatment of predilected lymphatic spread area for each type of head and neck tumor individually with a preservation of non-lymphatic structures of the neck. In case of a negative – N0 clinical finding on the neck we use perioperative biopsy of lymph node in a predilected area. When the biopsy is negative we continue with a selective neck dissection only of the predilected lymphatic area. When the biopsy is positive, or in case of a clinically apparent metastatic disease, we continue with a selective neck dissection of levels I–V. If the primary tumor extends beyond the midline, or in an advanced stage of the disease (clinically bilateral N+ finding), we approach to a bilateral selective neck dissection.

The surface size of a tumor does not correlate with the incidence of metastasis, therefore, the consideration of the patient's T category might not be sufficient for making optimal treatment decisions (11,12).

In our retrospective study within period from 1st January 2015 to 31th December 2019 we evaluated 396 patients who had been treated at our Clinic for an oncologic disease. In this group of patients we evaluate the location of the primary tumor, the type of block neck dissection, the separation quality of individual areas of lymphatic tissue, and the histopathology report.

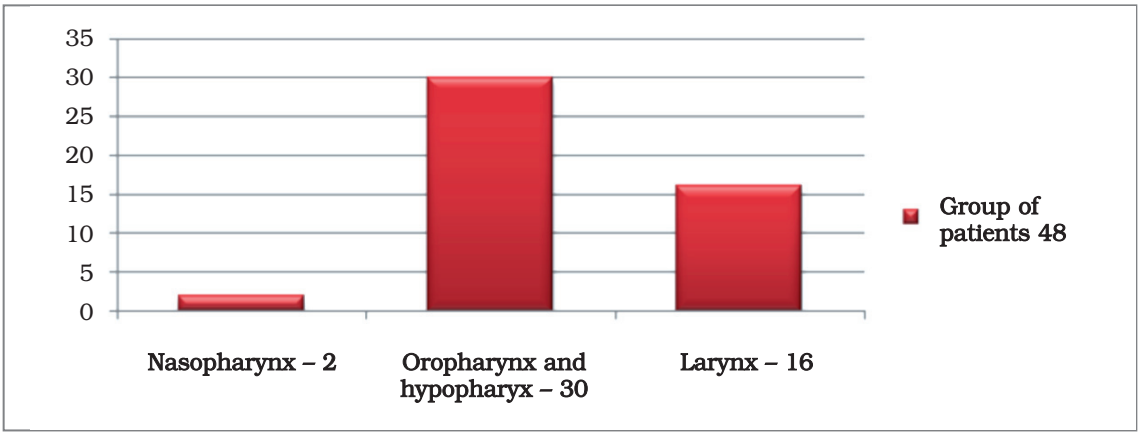

Fig. 2 Group of patients in study

48 patients were included in the study, in which a neck block dissection was performed with a thorough separation of adipo-lymphatic tissue according to the neck levels I–V, and a nodal metastasis of the primary tumor was confirmed.

The remaining 348 patients were excluded from the study due to a negative (N0) histopathology report or because of the inability to determine exactly in which group the metastatic lymph nodes were present due to an insufficient lymphatic tissue separation during the surgery.

RESULTS

The study population comprised of 45 males (93,7%) and 3 females (6,2%).The age of the patients ranges from 18-90 years. In 34 cases (70,8%) nodal metastases were clinically apparent or highly suspected, in the rest of them (29,2%) there was N0 finding.

1) In the group with nasopharyngeal cancer we had 2 patients, in both cases MTS were confirmed in the predicted area II. In addition, both had a nodal metastasis in area III, too.

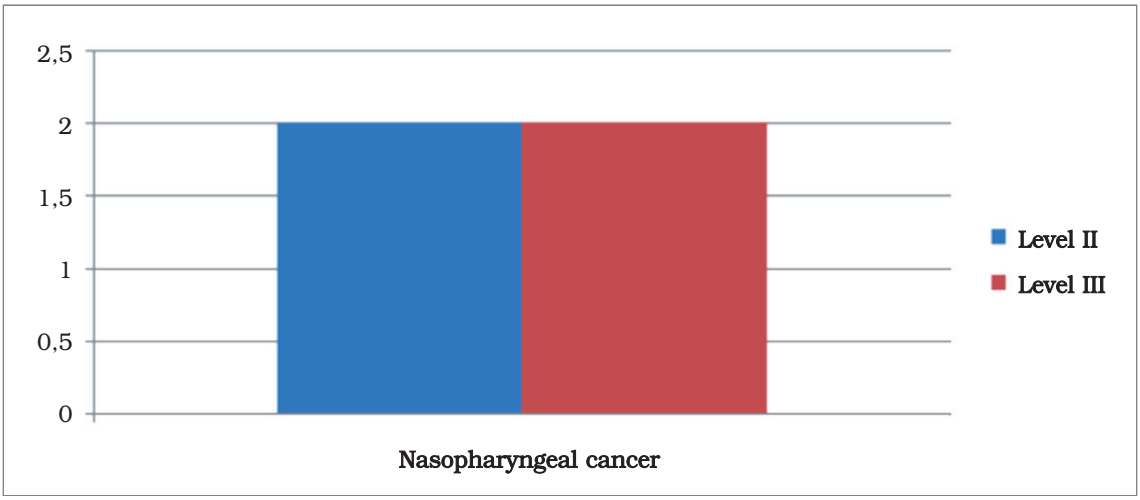

Fig. 3 Nodal MTS in nasopharyngeal cancer

2) We had 30 patients with confirmed MTS of oropharyngeal and hypopharyngeal tumors. 29 patients had a histologically verified metastasis in the predilected area II, III, or IV. Two of these patients had also a MTS in the area V. Additionally, 3 patients - 2 with carcinoma of the tongue root and 1 with carcinoma of the uvula had MTS bilaterally, which also corresponds to the data from literature.

Differences in the distribution of MTS were observed in 1 patient with a tongue root carcinoma in whom a MTS was histologically verified in the level Ib.

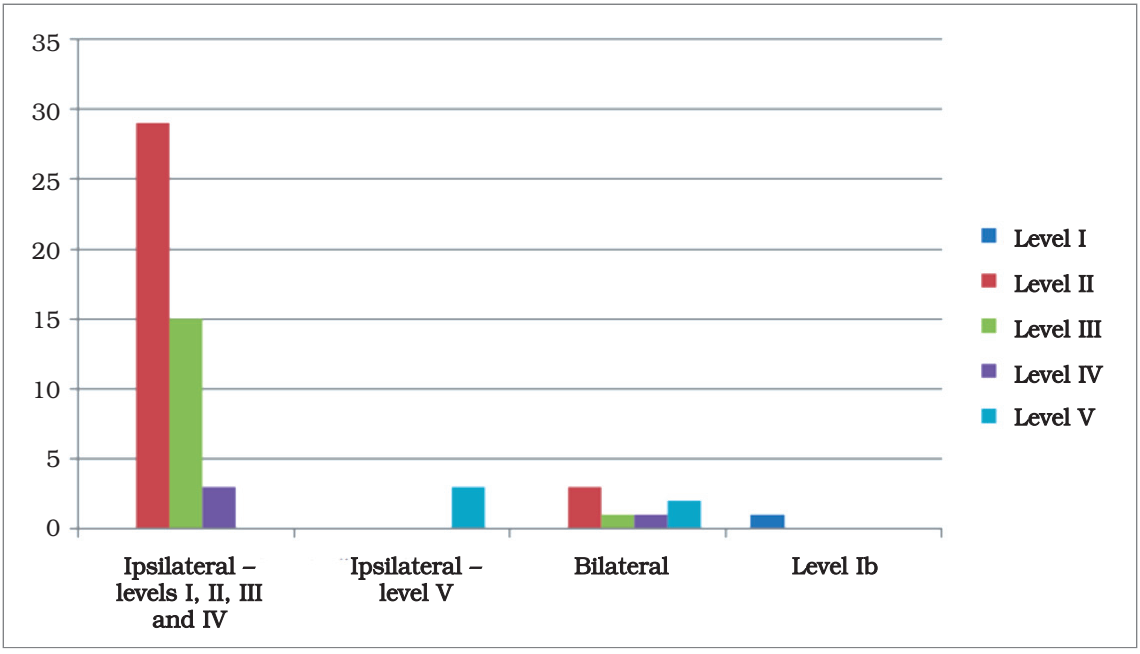

Fig. 4 Nodal MTS in oro-, and hypopharyngeal cancer

3) In the third group, we had 16 patients with laryngeal cancer, of which 2 were glottic and 14 supraglottic. In all patients there were nodal metastases in the predilected lymphatic area.

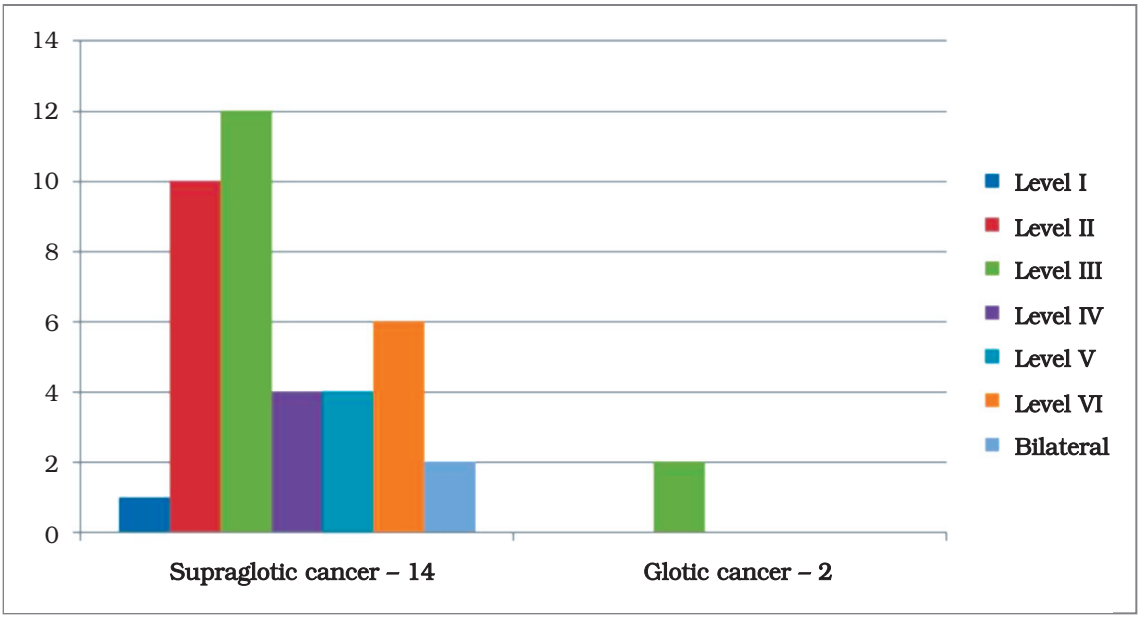

Fig.5 Nodal MTS in laryngeal cancer

DISCUSSION AND CONCLUSIONS

Based on literature and practice we know that the occurrence of nodal metastases is one of the most important prognostic factors in head and neck cancer. Early diagnosis is crucial for proper treatment. Unfortunately, the detection of early metastases remains a major challenge despite the constant development of imaging modalities (7). Whereas predictive diagnostic methods have they own limitation,at Clinic we decided to prefer a selective neck dissection based on a surgical treatment of predilected lymphatic spread area for each type of head and neck tumor individually with a preservation of non-lymphatic structures of the neck.

To confirm the correctness of our practice and to question the sentinel lymph node method we did a retrospective study.

We evaluated patients who had been treated at our Clinic for an oncologic disease and who had undergone a neck block dissection and who had a nodal metastasis of the primary tumor confirmed.

The main goal was to analyze the distribution of neck metastases and to study the frequency of skip metastases in head and neck cancer.

In our group of patients we confirmed a skip metastasis in 2.1% of cases (3.3% in pharyngeal carcinomas). The low incidence in our cohort was probably caused by an inaccurate separation of the dissected adipo-lymphatic tissue according to the levels I–V during the surgery or, in the case of extensive N3 metastases, the inability to exactly determine in which group the original lymph nodes were present.

Also the size of the group of patients itself is a possible cause of the low incidence of skip metastases.

At present we have adopted precise rules according to which the separation of dissected adipo-lymphatic tissue in elective and therapeutic cervical dissections will proceed in the future. After a sufficient enlargement of the group of patients we will evaluate the occurrence of skip metastases again.

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CLINICOPATHOLOGICAL STUDY OF SKIN ADNEXAL TUMORS: A SINGLE INSTITUTE EXPERIENCE

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Abstract

Background: Skin adnexal tumors (SATs) encompass a very broad variety of rare dermatopathologic entities.

Objective: The aim of the present study was to analyze an incidence and clinicopathological findings of patients with biopsy-proven ASTs.

Material and Methods: A retrospective review of all consecutive cutaneous tumors that were diagnosed at the Martin Biopsy Center in Martin from July 2019 to March 2021 was carried out. ASTs have been searched for from this file and studied based on their histologic type and line of differentiation, anatomic distribution, age, and gender.

Results: *Among* over 3,700 skin tumors a total of 38 ASTs in 38 individuals (21 males, 17 females) have been found. The age of the patients ranged from 22-89 years (mean 55.5 y.). The head and neck region was found to be the most common site affected (26/38; 68.4%), followed by extremities (8/38; 21.1%) and trunk (4/38; 10.5%). Tumors of hair follicle origin constituted the largest category (22/38; 57.9%), followed by sweat gland tumors (15/38; 39.5%) and tumors derived from sebaceous glands (1/38; 2.6%). Benign lesions accounted for all 38 cases. Trichoepithelioma was the most frequent lesion found in the category of follicular tumors and poroma was the most common among tumours with sweat gland differentiation.

Conclusion: An overall incidence of ASTs is low and in this institutional study they constituted about 1% of all cutaneous neoplasms. ASTs display a marked phenotypic heterogeneity, that is why many published studies have provided divergent results concerning their clinicopathological features.

Key words: skin adnexal tumors, trichoepithelioma, poroma, pilomatrixoma

INTRODUCTION

Skin adnexal tumors (SATs) encompass a very broad variety of rare dermatopathologic entities (1-3). Based on histogenetic origin they are classified into tumors with differentiation towards hair follicle, eccrine or apocrine sweat glands, and sebaceous glands (1–3). SATs principally arise from undifferentiated pluripotent stem cells present in the epidermis or appendageal structures that finally differentiate to specific tumors (4–6). This neoplastic transformation is a multifactorial process which is related to genetic background, activation of particular molecular pathways, epithelial-mesenchymal interplays, and local tissue microenvironment (5–7). That is why ASTs may sometimes display more than one line of appendageal differentiation, making precise classification of these neoplasms difficult (1–4). ASTs are generally infrequent and the vast majority of them are benign lesions without any prognostic impact (1–4). However, particular types of ASTs have got importance because they may herald complex hereditary syndromes and significant association with certain visceral malignancies (Table 1). Therefore, a correct diagnosis is essential to alert the clinicians to the possibility of these conditions and may have important therapeutic implica-

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tions. ASTs have a peculiar clinical manifestation, they can occur as solitary lesions or with multiple papules, nodules, or plaques (1, 2). Syndromic cases of ASTs are usually multiple (1). Dermoscopy does not reveal pathognomonic features and in most cases diagnosis is possible only on the basis of microscopic investigation (1). Although many lesions show remarkable variability in histological appearance, there is a considerable morphological overlap between individual entities (1). ASTs differ greatly not only in histomorphology but also in clinical presentation including age and gender preponderance and body site distribution. In this journal a unique case of a benign follicular tumor – trichoepithelioma with malignant transformation into basal cell carcinoma has been recently published by Bartoš (8). To the best of the author's knowledge, no original research evaluating a large series of AST cases has been published in Slovak medical literature until now. The aim of the present study was to analyze the incidence, spectrum, and clinicopathological findings of patients with biopsy-proven ASTs in a single pathology diagnostic centre. Own observations were then compared with the results of other papers.

MATERIAL AND METHODS

A retrospective review of all consecutive primary cutaneous tumors that were histologically diagnosed by the author of this article (V.B.) at the Martin Biopsy Center in Martin from July 2019 to March 2021 was carried out. The participants were registered in the Pathology Archive Computer Program, from which the required histopathological data were extracted. The analyzed cases comprised both benign and malignant neoplasms, including common melanocytic nevi and seborrheic keratoses. After completion the ASTs have been searched for from this file. Clinical data of the patients needed for the study were obtained from their medical records. Biopsy samples were processed in the formalin-fixed, paraffin-embedded tissue sections which were stained with hematoxylin and eosin and examined under a light microscope. Most cases of ASTs were also investigated with special staining and immunohistochemical methods as needed in specific situations. Tumors were analyzed based on their histologic type and line of differentiation, anatomic distribution, age, and gender.

RESULTS

Within the study period the author has investigated over 3,700 various skin tumors. Among them a total of 38 cases (1.02%) of ASTs in 38 individuals have been found. There were 21 males and 17 females with the male to female (M/F) ratio of 1.2 : 1. The age of the patients ranged from 22-89 years (mean 55.5 y.). Overall most common age groups affected were between 31 and 50 years (29.0%) and between 51 and 70 years (29.0%). The tumors of hair follicle origin constituted the largest group comprising 57.9% (22/38) of cases. It was followed by sweat gland tumors (15/38; 39.5%). The tumors derived from sebaceous glands accounted for only a single case (2.6%) (Table 2). Sweat gland tumors were more prevalent in males (F/M ratio of 1.5 : 1), while in follicular tumors the proportion of males and females was equal (F/M ratio of 1 : 1). The topographical distributions of lesions were diverse. The head and neck region was found to be the most commonly affected site (26/38; 68.4%), followed by extremities (8/38; 21.1%) and trunk (4/38; 10.5%) (Table 3). In the head and neck region, 14 reported cases were from facial and 12 cases from extrafacial parts. In the trunk and limbs, 3 cases were from back, 2 cases from forearm, and 1 each from chest, arm, thigh, shank, foot dorsum, hand dorsum, and finger. Benign tumours accounted for all 38 cases. No malignant neoplasia has been recorded. Trichoepithelioma was the most frequent lesion found in the category of follicular tumors and poroma was the most common among tumours with sweat gland differentiation (Table 4). Histopathology of some types of ASTs is illustrated in Figures 1, 2, and 3.

Table 1 Most common hereditary syndromes associated with ASTs. (summarized from ref. 1 and 2)

Syndrome	Observed AST	Another important findings
Cowden syndrome	trichilemmoma	breast, renal and endometrial cancers, palmoplantar keratosis
Muir-Torre syndrome	sebaceous tumors	colorectal, genitourinary, breast, and hepatobiliary malignancies
Gardner's syndrome	pilomatrixoma	gastrointestinal, liver, thyroid, bone, and brain malignancies
Rombo syndrome	trichoepithelioma	cutaneous basal cell carcinomas
Brooke-Spiegler syndrome	trichoepithelioma spiradenoma cylindroma	specific phenotypic form of disease is a multiple familial trichoepithelioma variant
Birt-Hogg-Dubé syndrome	fibrofolliculoma trichodiscoma	thyroid and renal cancers
Bazex-Dupré-Christol syndrome	trichoepithelioma	cutaneous basal cell carcinomas

Table 2 Age incidence distribution of ASTs observed in the present study

Tumor origin	N	≤ 30 y.	31 – 50 y.	51 – 70 y.	≥ 71 y.
Hair follicle	22	4 (18.1%)	6 (27.3%)	6 (27.3%)	6 (27.3%)
Sweat glands	15	2 (13.4%)	5 (33.3%)	5 (33.3%)	3 (20.0%)
Sebaceous glands	1	0	0	0	1 (100%)
Total	38	6 (15.7%)	11 (29.0%)	11 (29.0%)	10 (26.3%)

Table 3 Topographic distribution of ASTs observed in the present study.

Tumor origin	N	Head & neck	Trunk	Limbs
Hair follicle	22	16 (72.7%)	1 (4.6%)	5 (22.7%)
Sweat glands	15	9 (60.0%)	3 (20.0%)	3 (20.0%)
Sebaceous glands	1	1 (100%)	0	0
Total	38	26 (68.4%)	4 (10.5%)	8 (21.1%)

Table 4 Frequency of individual histologic types of ASTs observed in the present study

Tumor origin	Diagnosis	N	Male	Female
Hair follicle	Pilomatrixoma	7	4	3
	Classic trichoepithelioma	9	4	5
	Desmoplastic trichoepithelioma	1	0	1
	Trichoadenoma	1	0	1
	Trichofolliculoma	3	2	1
	Pilar sheath acanthoma	1	1	0
Sweat glands	Poroma	7	5	2
	Hidradenoma	1	1	0
	Hidrocystoma	1	0	1
	Syringocystadenoma papilliferum	1	0	1
	Tubular adenoma	3	2	1
	Spiradenoma	2	1	1
Sebaceous glands	Sebaceous adenoma	1	1	0

Table 5 Prevalence of three main histogenetic groups of ASTs based on individual studies (4–7, 9–17). *among a total of 66 ASTs there were two mixed tumors which were not calculated

	Total number	Sweat gland tumors		Hair follicle tumors		Sebaceous gland tumors	
ref. 4	1,016	215	(21.2 %)	265	(26.1%)	536	(52.7%)
ref. 7	110	41	(37.3 %)	43	(39.1 %)	26	(23.6 %)
ref. 11	56	24	(42.9 %)	20	(35.7 %)	12	(21.4 %)
ref. 10	96	43	(44.8 %)	49	(51,0 %)	4	(4.2 %)
ref. 5	33	19	(57.6 %)	12	(36.4 %)	2	(6.0 %)
ref. 12	25	14	(56.0 %)	7	(28.0 %)	4	(16.0 %)
ref. 6	66 (64)*	20	(31.2 %)	25	(39.1 %)	19	(29.7 %)
ref. 17	60	27	(45.0 %)	14	(23.3 %)	19	(31.7 %)
ref. 13	35	17	(48.6 %)	11	(31.4 %)	7	(20.0 %)
ref. 14	25	13	(52.0 %)	8	(32.0 %)	4	(16.0 %)
ref. 15	30	20	(66.7%)	8	(26.7 %)	2	(6.6 %)
ref. 16	51	22	(43.1%)	19	(37.3%	10	(19.6%)
ref. 9	52	41	(78.8%)	4	(7.7%)	7	(13.5%)
this study	38	15	(39.5%)	22	(57.9%)	1	(2.6%)

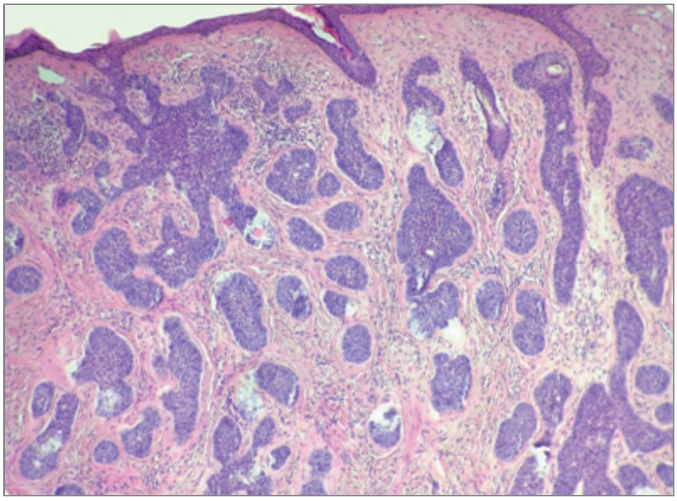

Fig. 1 Classic trichoepithelioma. The tumor is composed of small irregular basophilic lobules within a dense fibrous stroma. (hematoxylin & eosin, magnification 20x)

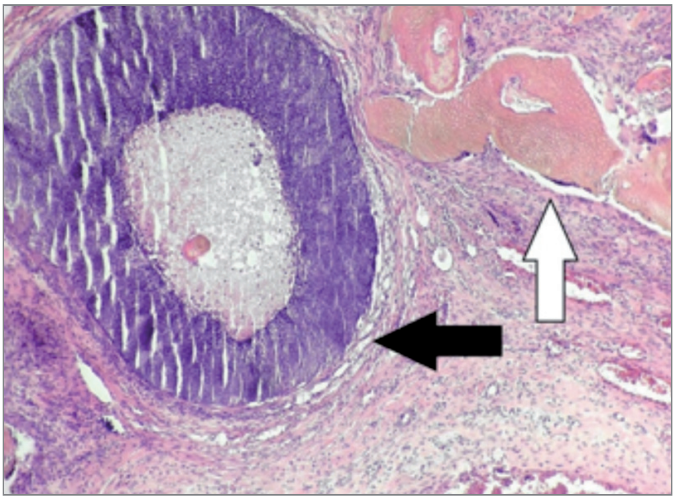

Fig. 2 Pilomatrixoma. The typical biphasic population composed of germinative basaloid cells (black arrow) and eosinophilic ghost cells (white arrow). (hematoxylin & eosin, magnification 20x)

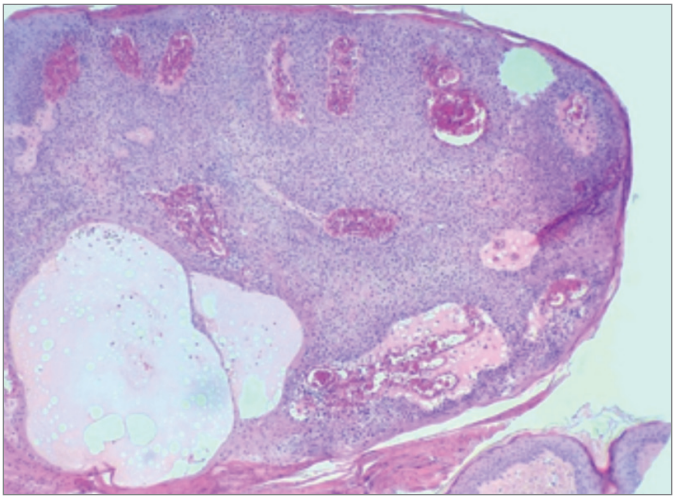

Fig. 3 Polypoid eccrine poroma. The confluent downward proliferation of the poroma cells with cystic lumina within the tumor. (hematoxylin & eosin, magnification 10x)

DISCUSSION

In global literature there is a lack of relevant large-scale epidemiologic studies that are specifically focused on appendageal skin neoplasms. As a consequence no reliable data exist on the geographic and racial incidence of these tumors. In this research the histopathological prevalence of ASTs among over 3,700 consecutive cutaneous tumors was 1.02%. This was similar compared with the study of Samaila (9) who found that ASTs accounted for 0.9% of all cutaneous neoplasms. Although ASTs constitute a very small proportion of skin tumors in our setting, the true incidence is probably higher than reported because many ASTs are asymptomatic benign lesions that are not troublesome and do not have to be surgically removed.

In the present study the most common line of histogenetic differentiation encountered was follicular differentiation, followed by sweat gland differentiation and by far the least frequent being sebaceous differentiation. These observations are in concordance with that of Suri et al. (6), Kaur et al. (7), and El Ochi et al. (10). Other authors (5,11-16) have reported sweat gland tumors to be the most common, followed by hair follicle tumors. Interestingly, Kamyab Hesari et al. (4) found sebaceous tumors to be the most frequent type, which is unlike of most published studies. This is, however, easily explained because the authors included into this category also sebaceous nevus of Jadasson and sebaceous hyperplasia which are not true neoplasias. In the study of Samaila (9) and Sahu et al. (17) the tumors derived from hair follicle were the least common of all cases. As shown in the Table 5, the percentages of three main varietes of ASTs vary significantly depending on given studies. Even a spectrum of individual histologic types has been very different. As regards hair follicle and sweat gland tumors, the most common types confirmed in our study were trichoepithelioma and poroma, respectively. Other authors have found them as follows: pilomatrixoma & hidradenoma (7,10,11,15), pilomatrixoma/trichoepithelioma & hidradenoma (16), trichoepithelioma & syringoma (5), pilomatrixoma & hidradenoma papilliferum (12), pilomatrixoma & spiradenoma (6), pilomatrixoma & poroma (17), and trichoepithelioma & acrospiroma (9).

In accord with previous papers we have shown an evident predominance of ASTs in the head and neck region. This is accounted for by the fact that this body site is rich in pilosebaceous units and sweat glands, thereby providing a fertile environment for the development of ASTs. However, the proportional involvements of this area differ markedly (46% – 88%) among analyses conducted in various countries (4–7, 9–17).

Concerning age, this study has demonstrated that benign SATs show a very wide range of age distribution. The mean age was 55.5 years while a maximum number of cases were found within the age category of 31 – 50 years and 51 – 70 years. These values are somewhat higher compared to most published studies. Many authors have confirmed the average age between 32 – 36 years (4, 9, 10, 12, 15) and the most frequent occurrence in people aged between 20 and 40 years (6, 7, 10, 12, 13, 17). From the above data one might have concluded that SATs develop most often in younger persons in the third and fourth decades of life. However, several papers have reported data outside of this range. In the study of Nair et al. (5) the tumors were most common in patients under the age of 20, whereas in the research conducted by Sharma et al. (11) they were most frequently found between 51 – 60 years. Further, some investigators have noted a bimodal age distribution with two peaks, i.e. between 31 – 40 years and 51 – 60 years (15), or up to 20 years and then in the fifth decade (9).

As for gender, many published papers have documented (4, 7, 9, 11, 12, 15) that both sexes are roughly equally affected. Some authors have described a slightly higher preponderance of males (6, 10, 17), while others have reported a mild predominance of females (5, 13, 14, 16). These discrepancies must have been related to disparate proportions of histologic types of tumors in the study cohorts. It has been well-known that many ASTs develop more commonly at a certain age and affect more frequently a certain gender. For example, trichofolliculoma and chondroid syringoma have a predilection for middle-aged

men (1). Tubular adenoma most frequently occurs in middle-aged women and sebaceoma in elderly women (1). Melanocytic matricoma has a predilection for elderly men (1). Classic syringoma often occurs in elderly women but its eruptive vatiant is more frequent in young women (1). Syringocystadenoma papilliferum is more common in females and myoepithelioma more frequent in males (1). Hidradenoma papilliferum almost exclusive affects women (1). Hence it is obvious that precise analyzes of demographic data and clinicopathologic findings of ASTs must to take into account their individual histological subtypes and varieties, some of which should be better evaluated separately.

It is also important to note that ASTs, especially their malignant forms, often histologically mimic cutaneous metastases and vice versa (18). Morphologic distinction between primary AST and metastatic malignancy can be very difficult but it has a key impact on further diagnostic and therapeutic management of the patient. The lesions that are sometimes problematic to differentiate are e.g. clear cell nodular hidradenoma vs metastatic clear cell renal cell carcinoma, high-grade porocarcinoma vs metastatic poorly differentiated non–small cell carcinoma or high-grade transitional cell carcinoma, low-grade ductal carcinoma of the skin adnexa vs metastatic low-grade ductal carcinoma of the breast or salivary gland, and basaloid adnexal carcinoma vs metastatic breast carcinoma (18). The subject matter of their differential diagnosis is very complex and this topic would go far beyond the scope of this article. In general, the combination of epidermal involvement, absence of multifocality, lack of Grenz zone, and absence of lymphovascular invasion favours primary malignant AST rather then metastasis (18). Although immunohistochemistry has a limited value in the exact categorization of ASTs, it may help to distinguish primary ASTs from metastatic cancers. Sariya et al. (18) have found that a simultaneous using of four immunohistochemical markers (p63, B72.3, calretinin, CK5/6) may be of help in this regard. Whereas p63 and CK5/6 are positive in virtually all SATs, they are negative in the vast majority of metastatic adenocarcinomas. SATs are usually reactive for calretinin and negative for B72.3, while metastatic cancers are generally positive for B72.3 and negative for calretinin. Anyway, many problematic cases require a thorough clinicopathological correlation and an extensive clinical evaluation is usually necessary to exlude metastasis from elsewhere.

CONCLUSION

An overall incidence of ASTs is low and in this institutional study they constituted about 1% of all skin neoplasms. They were prevalent in middle-aged group with a slight predilection for men, the head and neck region was the most frequently affected and the hair follicle tumors represented the most common histogenetic group. ASTs manifest a striking phenotypic heterogeneity, that is why many published studies have shown diverse results regarding their clinicopathological features.

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HOW TO MEASURE PATIENT SAFETY CULTURE? A LITERATURE REVIEW OF INSTRUMENTS

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Abstract

Introduction: Patient safety culture is described as employees' shared values, attitudes, and behaviours in a healthcare organization. Its main goal is to improve patient safety. Assessment of patient safety culture in the hospital environment is most often carried out using self-assessment tools. Although several of these tools have been developed, their comprehensive overview is lacking in literature.

Aim: To provide an overview of instruments measuring patient safety culture in a hospital setting.

Methods: The study has a character of a narrative literature review. The search was performed in the scientific databases Scopus, ProQuest, and PubMed in January 2021. The search produced a total of 1,767 studies and was limited to language (English). The search and the retrieval process reflected PRISMA's recommendations. The content analysis method was used in the data synthesis.

Results: We identified 24 tools for assessing the patient safety culture in a hospital setting, of which seven were developed for specific workplaces; others are considered general. Eighteen tools might be utilized by all healthcare professionals within the hospital setting and only three were designated explicitly for nurses. The most commonly used instruments were the Hospital Survey on Patient Culture and the Safety Attitudes Questionnaire.

Conclusion: Assessing a patient safety culture is considered one of the strategies for improving patient safety while increasing care quality. An appropriate tool's choice depends on the target population, the instrument's validity and reliability, and other aspects. Awareness of the various assessment tools can help hospitals choose the one that best suits their circumstances.

Keywords: Hospital; Instrument; Nurse; Patient safety culture; Safety climate

INTRODUCTION

A patient safety culture assessment is important for improving patient safety within healthcare delivery, especially for identifying additional risks and threats before causing a real problem (1). The concept of safety culture represents the organizational culture in which employees want to provide the safest possible healthcare. Effective evaluation of patient safety culture embodies the regular utilization of self-assessment instruments by healthcare professionals. These instruments are constructed to explore patient safety attitudes, identify aspects of care requiring emergent attention, and motivate hospital management to plan strategies targeted at decreasing general risks that threaten patient safety (2). In many countries, patient safety culture assessments are required by accreditation committees. Several instruments were developed to fulfil these requirements (3).

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However, in the Slovak Republic and the Czech Republic conditions, patient safety culture assessments are voluntary and none of the existing instruments was utilized as mandatory in clinical practice. Despite this, healthcare organizations should carefully consider the right choice of an instrument measuring patient safety culture. Nowadays, there are too many instruments measuring patient safety culture and others are being developed. Also, it is not entirely clear which specific tools are available for its utilization. It is not very clear whether any of them can be applied to a particular workplace's conditions. Another issue that needs to be considered is the intended study population. Few instruments are suitable only for nurses as the most prominent group of healthcare professionals. According to the review published within the American context, thirteen instruments were developed for measuring patient safety culture in different settings (4). Recently, a review published by Australian researchers revealed nine instruments that might be used during healthcare organizations' accreditation (5). Both reviews offer valuable information on particular instruments; however, none of these provides a complex overview of instruments that might be used within the hospital setting. Therefore, our study aimed to provide a complex overview of instruments measuring patient safety culture in a hospital setting.

METHODS

The paper has adopted the design of a narrative literature review. The literature search was performed in three scientific databases – Scopus, ProQuest, and PubMed in January 2021 (from earliest to January 2021). The databases were selected based on their institutional availability. The literature search was carried out using the exact keywords and Boolean operators AND and OR in all databases as follows hospital, instrument, nurse, patient safety culture, safety climate. The search was limited to language (English) and peer-review papers. The search was not limited to a time period. According to the predefined criteria, we included studies that were: a) quantitative empirical papers (validation studies), b) published in peer-review journals, c) written in English language, d) involving nurses, and e) focused on the topic of interest – measuring patient safety culture in a hospital setting. We excluded studies that were: a) qualitative or mixed-method empirical papers and b) editorials, reviews, case studies, discussion papers or protocols, and c) focusing on hospital management. Based on these criteria, the literature search produced 1,767 studies (519 in Scopus, 849 in ProQuest, and 399 in PubMed). Additional six studies were added to the total number of studies based on the manual search of reference lists from included studies. The total of studies included in the analysis was 1,773. The search and the retrieval process reflected PRISMA's recommendations (Figure 1). The data were systematically retrieved by two independent researchers (DB, DK) within two phases. We used the program Rayyan QCRI® in both retrieval phases (6). After removing duplicates (n=415), 1,358 papers were analyzed by reading titles, abstracts, and inclusion criteria in the first phase. Within the second phase, we examined a total of 26 studies by reading full texts. An agreement between two independent researchers was achieved, and four papers were excluded for a reason (insufficient information about instruments). A total of twenty-four studies were included in the final analysis. The data from twenty-four studies were extracted by two researchers using a spreadsheet: author, year, country, instrument, number of items, dimensions of patient safety, evaluation description, and intended study population. The synthesis of the data was performed in a narrative and tabular way of processing. The data were analyzed using the summative content analysis method (7).

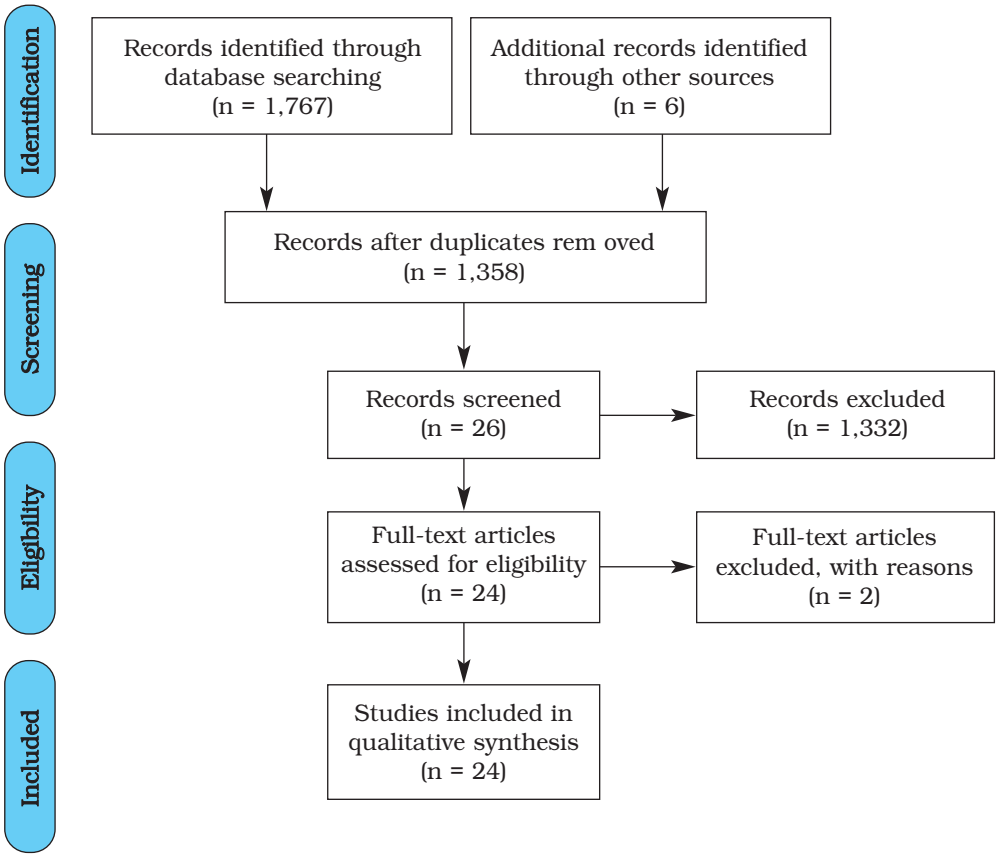

Fig. 1 Flow diagram – recommendation PRISMA

RESULTS

Survey characteristics

Twenty-four instruments suitable for utilization in a hospital setting were identified in our review. A significant proportion of studies included in the review were conducted in the USA (n = 15; 2 instruments from AHRQ). Studies were also conducted in Australia, Canada, Japan, and in European countries, such as Italy, Germany, and UK. Seventeen instruments are considered general (Table 1) and seven instruments were developed for specific care units (Table 2). Instruments for specific care units were for high-risk hospital areas (12), operating rooms (13), obstetrics care units (14), intensive care units (17, 24), acute geriatric units (22), and for any phases of perianesthesia (29). Of all instruments, eighteen might be utilized by all healthcare professionals within the hospital setting and three instruments are suitable for use by nurses (21, 26, 29), one by physicians, nurses and pharmacists (11), and two by physicians and nurses (22,27).

The number of items ranges from 9 (26) to 128 (22) in the tools mentioned above. For example, the lowest number reflecting dimensions of patient safety culture is 3 in the Short-Form Patient Safety Climate in Healthcare Organisations instrument (9). The individual dimensions in identified instruments covered the important categories reported by Singla et al. (4) as follows: Management/Supervision, Safety system, Risk, Work pressure, Competence, Procedures/Rules, Additional dimensions. The highest number of dimensions is 23, reported in the Patient and Occupational Safety Culture Questionnaire (27), the lowest then

Table 1 Overview of general instruments measuring patient safety culture in hospital settings

Author, year, country	Instrument	Number of items	Dimensions of patient safety	Evaluation description
Agency for Healthcare Research and Quality (1, 8) USA	Hospital Survey on Patient Safety Culture 1.0 Hospital Survey on Patient Safety Culture 2.0	42 34	12 10	5-point Likert scale; an open-ended section for comments. The same as in version 1.0.
Benzer et al. (9) USA	Short-form Patient Safety Climate in Healthcare Organisations	15	3	*Not reported*
Ginsburg et al. (10) Canada	Modified Stanford Instrument (MSI-06)	32	5	*Not reported*
Itoh et al. (11) Japan	Questionnaire-based Survey of Safety Culture	57	9	5-point Likert scale
Petschonek et al. (15) USA	Just Culture Assessment Tool	27	6	7-point Likert scale
Pronovost et al. (16) USA	Safety Climate Scale (SCS)	10	*Not reported*	5-point Likert scale
Sexton et al. (17) USA	Safety Attitudes Questionnaire (SAQ)	60	6	5-point Likert scale
Sexton et al. (18) USA	Safety, Communication, Operational Reliability and Engagement survey (SCORE) Survey	48	8	5-point Likert scale
Singer et al. (19) USA	Stanford/PSCI Culture Survey	30	16	5-point Likert scale; dichotomous response options
Singer et al. (20) USA	Patient Safety Climate in Healthcare Organisations (PSCHO)	38	9	5-point Likert scale
Stevanin et al. (21) Italy	The Multidimensional Nursing Generations Questionnaire	54	8	5-point Likert scale
Thomas et al. (23) USA	Safety Climate Survey (SCSu)	21	*Not reported*	5-point Likert scale
Victorian Managed Insurance Authority (25) Australia	Victorian Safety Climate Scale	74	6	5-point Likert scale
Vogus and Sutcliffe (26) USA	The Safety Organizing Scale	9	1	*Not reported*
Wagner et al. (27) Germany	Patient and Occupational Safety Culture Questionnaire	73	23	5-point Likert scale
Weingart et al. (28) USA	Culture of Safety Survey	34	5	5-point Likert scale; dichotomous response options

is only one for The Safety Organizing Scale (26). In three instruments there were no identified dimensions (Table 1, 2).

Internal consistency for the identified instruments ranges from 0.50 to 0.94. However, for most tools, these values have been given for individual dimensions, but not for the whole tool. No internal consistency values were stated for ten instruments (Table 3).

Table 2 Overview of specific instruments measuring patient safety culture in hospital settings

Author, year, country	Instrument	Number of items	Dimensions of patient safety	Evaluation description
Kaissi et al. (12) USA	Teamwork and Patient Safety Attitudes Questionnaire	24	4	5-point Likert scale
Makary et al. (13) USA	Safety Attitudes Questionnaire – Operating room	30	6	5-point Likert scale
Milne et al. (14) UK	Cultural Assessment Survey	37	6	*Not reported*
Sexton et al. (17) USA	Safety Attitudes Questionnaire – Intensive Care Units (SAQ–ICU)	65	6	5-point Likert scale, an open-ended section for comments.
Steyrer et al. (22) Germany	Patient Safety Culture Questionnaire	128	8	*Not reported*
Thomas and Lomas (24) UK	Safety Attitudes Questionnaire – ICU Short Form	37	10	5-point Likert scale
Windle et al. (29) USA	Perianesthesia Safe Practices Instrument	65	*Not reported*	5-point Likert scale

Table 3 Validity and internal consistency through Cronbach´s alpha values for individual instruments

Instrument	Internal consistency (Cronbach alpha)	Validity
Hospital Survey on Patient Safety Culture 1.0 (1)	0.63 to 0.84	Construct validity (EFA* - 12 factors)
Hospital Survey on Patient Safety Culture 2.0 (8)	Not reported	Not reported
Patient Safety Climate in Healthcare Organisations (20)	0.50 to 0.89	Construct validity (EFA – 7 factors; multitrait analysis – 9 factors)
Short-form Patient Safety Climate in Healthcare Organisations (9)	0.74 to 0.84	Construct validity (CFA** – 3 factors)
Stanford/ PSCI Culture Survey (19)	Not reported	Construct validity (EFA – 5 factors)
Modified Stanford Instrument (10)	0.81 and 0.88	Construct validity (EFA – 5 factors)
Questionnaire-based Survey on Safety Culture (11)	Not reported	Not reported
Teamwork and Patient Safety Attitudes Questionnaire (12)	0.62 to 0.87	Construct validity (EFA – 4 factors)
Safety Attitudes Questionnaire (17)	0.90	Construct validity (CFA – 6 factors)
Safety Attitudes Questionnaire – Intensive Care Units (17)	0.90	Construct validity (CFA – 6 factors)

Safety Attitudes Questionnaire – Intensive Care Units – Short Form (24)	0.90	Construct validity (CFA – 6 factors)
Safety Attitudes Questionnaire – Operating room (13)	0.76	Content validity (review of literature, review of the survey by OR healthcare providers, focus groups); construct validity (CFA – 7 factors)
Cultural Assessment Survey (14)	0.72 to 0.84	Content validity (review of literature, focus groups, review of the survey by key informants)
Just Culture Assessment Tool (15)	0.63 to 0.86	Content validity (review of literature, survey review); construct validity (CFA – 7 factors)
The Safety Climate Scale (16)	0.68 to 0.81	Construct validity (CFA – 6 factors)
Safety Climate Survey (23)	Not reported	Content validity (focus groups); construct validity (single factor structure)
Safety, Communication, Operational Reliability and Engagement survey (18)	0.82 to 0.92	Not reported
The Multidimensional Nursing Generations Questionnaire (21)	0.60 to 0.84	Face validity (review by nurses); content validity (review of literature, expert panel); construct validity (EFA – 8 factors)
The Patient Safety Culture Questionnaire (22)	0.84 to 0.91	Content validity (review of literature, expert panel); convergent validity (adverse events); construct validity (EFA – 7 factors)
Victorian Safety Climate Scale (25)	Not reported	Not reported
The Safety Organizing Scale (26)	0.88	Content validity (review of literature, expert panel, pretest with a sample of 45 RNs); construct validity (CFA – single factor structure); discriminant validity (employee commitment, trust in manager)
Patient and Occupational Safety Culture Questionnaire (27)	0.59 to 0.89	Content validity (expert panel)
The Culture of Safety Survey (28)	Not reported	Face validity (survey review); content validity (review of literature, focus groups, survey review)
Perianesthesia Safe Practices Instrument (29)	0.79 to 0.94	Content validity (review of literature, expert panel)

*EFA – exploratory factor analysis; CFA – confirmatory factor analysis

Development of individual tools

HSOPS version 1.0 instrument was developed originally by Westat under a contract with AHRQ (1) based on the existing literature review on patient safety culture and a review of two instruments – Veterans Health Administration Patient Safety Questionnaire and the Medical Event Reporting System for Transfusion Medicine. HSOPS version 1.0 has been recently piloted by AHRQ (8) to *HSOPS version 2.0*, which is recommended instead of the first version.

Patient Safety Climate in Healthcare Organisations was developed by Singer et al. (20) based on five existing instruments. Items from these instruments were modified for use in a hospital setting. In 2017, a *short form of Patient Safety Climate in Healthcare Organisations* was published by Benzer et al. (9).

Stanford/ PSCI Culture Survey was developed by Singer et al. (19) based on five existing instruments – Management Attitudes Questionnaire, Anesthesia Work Environment Survey, Naval Command Assessment Tool, Risk Management Questionnaire, Safety Orientation in Medical Facilities. However, the instrument was modified to *Modified Stanford Instrument* (10).

A Questionnaire-based Survey on Safety Culture was developed by Itoh et al. (11) based on adopting four parts of the questionnaire from the Operating Team Resource Management Survey.

Teamwork and Patient Safety Attitudes Questionnaire was developed by Kaissi et al. (12) based on the extraction of items from previous questionnaires and expert opinions of local healthcare leaders and researchers.

Safety Attitudes Questionnaire (SAQ) was developed by Sexton et al. (17) based on the modification of the existing instrument – Intensive Care Unit Management Attitudes Questionnaire (23), which derived from the Flight Management Attitudes Questionnaire, and also discussions with healthcare providers and subject matter experts and two conceptual frameworks: Vincent's framework for analyzing risk and safety (30) and Donabedian's model of quality care (31). The tool was adapted for use in intensive care units (17) and operating rooms (13). Furthermore, the *Safety Attitudes Questionnaire – Intensive Care Units* was modified by Thomas and Lomas (24) to reflect UK practice concerning job roles and published as *Safety Attitudes Questionnaire – Intensive Care Units – Short Form*. *Safety Attitudes Questionnaire – Operating room* was adapted from the original SAQ instrument, and modifications were made based on a literature review on patient safety in the operating rooms, results of the focus groups, and a review of the questionnaire by operating room healthcare provider (13).

Cultural Assessment Survey was developed by Milne et al. (14) based on a literature review on patient safety publications and best practices within the health care environment and key informant interviews with members of the Managing Obstetrical Risk Efficiently Program of the Society of Obstetricians and Gynaecologists.

Just Culture Assessment Tool was developed by Petschonek et al. (15) based on a comprehensive review of the just culture literature and safety culture literature and existing patient safety culture measurements.

The Safety Climate Scale was developed by Pronovost et al. (16) based on the existing instrument – the Flight Management Attitudes and Safety Survey (23).

Safety Climate Survey was developed by Thomas et al. (23) and endorsed by the Institute for Healthcare Improvement based on the items from SAQ.

Safety, Communication, Operational Reliability, and Engagement survey (SCORE) was developed in 2014 by Sexton et al. (18) based on the update of SAQ to reflect contemporary healthcare safety needs.

The Multidimensional Nursing Generations Questionnaire was developed by Stevanin et al. (21) based on a systematic literature review and opinions from an expert panel.

The Patient Safety Culture Questionnaire was developed by Steyrer et al. (22) based on an extensive literature review on instruments measuring patient safety culture and qualitative interviews with health care experts.

Victorian Safety Climate Scale was developed by the Victorian Managed Insurance Authority (25) based on SAQ items; however, the specific work settings items were replaced with more general ones. As a result, the instrument was more relevant and applicable to Australian hospital settings.

The Safety Organizing Scale was developed by Vogus and Sutcliffe (26) based on a review of case studies of high-reliability organizations (HROs).

Patient and Occupational Safety Culture Questionnaire was developed by Wager et al. (27) based on dimensions from the German version of the HSOPS instrument and SAQ and literature review on occupational safety, including risk and prevention

The culture of Safety Survey was developed by Weingart et al. (28) based on a literature review focusing on safety culture, organizational culture, and high-reliability organizations, as well as on focus groups.

Perianesthesia Safe Practices Instrument was developed by Windle et al. (29) based on a literature review on tools measuring patient safety culture and a review of additional studies.

DISCUSSION

Creating a culture of patient safety is one of the critical challenges which healthcare organizations are facing nowadays. Recently, many hospitals have begun assessing safety culture and improving the overall quality of provided care. However, they do not often know which tools are available and which one they should choose when choosing (1–3). Therefore, the main aim of our review was to provide an overview of instruments measuring patient safety culture in a hospital setting. In the beginning of January 2021, we identified a total of twenty-four instruments and described their development and psychometric properties. Almost all instruments were developed within the Anglo-American context and only three tools were developed in European countries. However, all of the identified instruments reflect the sociocultural contexts of the Slovak or Czech clinical nursing practices. These instruments are suitable for utilization in both countries, with respect to translations and minor cultural adaptations related mainly to job titles or categories of healthcare personnel. All instruments are designed for a quantitative data processing. Some of these instruments reflect the specific requirements of the environment, respondent or other circumstances. In newly-developed instruments, the number of particular items related to patient safety culture is growing exponentially. Therefore, organizations have an opportunity to choose from a wide range of these instruments. However, selecting an appropriate tool is often not an easy process. The tool should show satisfactory results in a pilot survey of psychometric properties in the organization or country before its implementation in practice (2). Concerning psychometric properties, instruments showed different values of validity and reliability. Data on psychometric properties have not been published in four instruments yet. However, according to the obtained data on most instruments, we may conclude that these are valid and reliable.

Singla et al. (4) conducted the first review and synthesis of the measurement tools related to patient safety. They identified 13 of these instruments, including 657 questions related to patient safety. Most of the recognized instruments were also included in our review. However, the rest of the tools described in Singla's study were only included in unpublished personal communication. We do not consider this method of inclusion of instruments very appropriate. There might be hundreds of tools that have been developed, but their psychometric properties or development process have not been published. Therefore, we analyzed only primary sources in our review. Besides, instruments related exclusively to management were included in Singla's review. On the contrary, we excluded this type of instrument due to its specific focus and the fact they did not consider patient safety culture in general. The review of Singla et al. (4) also identified individual questions and grouped them into 23 dimensions of patient safety culture. A similar num-

ber of dimensions was reported in a recent questionnaire development study of Wagner et al. (27).

More recently, Hodgen et al. (5) conducted a review focusing on identifying instruments suitable for assessing patient safety culture during the accreditation processes under the Australian Health Service Safety and Quality Accreditation Scheme. Based on their results, none of the instruments was recognized to assess all the safety culture's main components, thus could not be implemented during the accreditation process. On a small sample of hospitals in Australia it has been found that the safety culture is assessed through internal ways (primarily by questionnaires) which are usually designed based on a shortlist of questions from some instruments, such as SAQ. In a review of safety culture assessment tools by Hodgen et al. (5), a total of nine instruments were examined. These instruments were included according to the frequency of their citations, validity, and other established criteria. One of the identified instruments was designated for a qualitative processing and due to its nature not included in our review. The other instruments listed and further analyzed in the study by Hodgen et al. (5), which used quantitative self-report measures, were consistent with these identified in our study.

In terms of the number of studies published in connection with the individual instruments, we may conclude that the most widespread, translated into various languages, and popular among researchers around the world are those reviewed by Sexton et al. (17) – the Safety Attitude Questionnaire (SAQ) and the Hospital Survey on Patient Safety Culture (HSOPS). These instrument have shown acceptable validity and reliability and are highly recommended for use in various sociocultural contexts. Both of them reflect the basic dimensions of patient safety, including teamwork, communication, and management support. The differences between them lie in including issues related to human rights and job satisfaction in the SAQ while involving issues concerning handoffs, transitions, and management support in HSOPS. Regarding the use of these instruments in the Czech Republic and Slovakia, only the HSOPS has been tested on a sample of registered nurses in the Czech and Slovak hospital environment (e.g. 32-35). However, HSOPS questionnaire was validated for the conditions of the Czech nursing practice revealing the same factor structure as original version (32). Recently, the validation study of the HSOPS was also conducted within the Slovak nursing practice; nevertheless indicating the eight factor structure (33). To the authors´ knowledge, other instrument measuring patient safety culture has not been used within these two countries. Still, based on the results of the studies mentioned above, the evaluation of patient safety culture with this tool seems to be generally acceptable in Czech and Slovak practice conditions. It supports the view of maintaining the uniform assessment structure recommended by AHRQ. Moreover, study results might be comparable among the countries on the international level by using the databases of studies recommended by AHRQ or authors of the SAQ. Even though the SAQ has not been used in the Czech or Slovak conditions but based on the results of psychometric testing of the SAQ, we recommend using both instruments in further studies concerning the patient safety culture assessment.

CONCLUSION

In our review we identified a total of 24 instruments for assessing the culture of patient safety. The particular tools differ in the number of items, the evaluation of various dimensions, the psychometric characteristics, the target group of respondents, or specific focus to certain workplaces. Nowadays, patient safety is a highly discussed issue internationally, especially its impact on the quality of care. However, we believe that through its regular and repeated assessment, management can focus directly on problematic areas related to patient safety terms. For this assessment, it is vital to determine which instrument will be utilized in the organizations in advance. Awareness of particular instruments and their differences may help organizations choose the one that will suit their circumstances.

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