ISSN 1335-8421 Acta Med Mart 2007, 7(2)

ACTA MEDICA MARTINIANA

Journal for Biomedical Sciences, Clinical Medicine and Nursing

Contents

3
Semi-interactive detection of action potentials using local wave features and clustering
Lukáš Tůma, Klára Bernášková, Jan Mareš

9
Tumour suppressive effect of letrozole in mammary carcinogenesis of female rats
Vladimíra Sadloňová, Peter Kubatka, Iveta Švecová, Karol Kajo, Gabriela Nosáľová, Jurina Sadloňová

13
Autoimmune thyroiditis and isohormonal therapy in childhood – 10 years follow up
Jozef Michálek, Gedeon Fodor, Eva Mendelová, Marián Šmoldas

16
The epidemiology of food atopy patch tests: a study of 335 unselected school children aged 10 from two European countries
Roberto Ronchetti, Miloš Jeseňák, Mario Barreto, Dagmar Trubačová, Zuzana Rennerová, Vladimír Pohanka, Peter Bánovčin, Maria Pia Villa

26
Blood borne infections in stomatological practice: Synthesis of answers according to the fields of interest based on the questionnaire focusing on estimation of blood borne infections risk in stomatological practice
Elena Nováková, Ivan Snopek, Pavel Hubočan, Dagmar Mullerová, Jana Kompaníková, Nils Skaug, Eugenia Aura Negut

Published by the Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia

Editorial Office:
Acta Medica Martiniana
Jessenius Faculty of Medicine, Comenius University
(Dept. of Physiology)

Malá Hora 4
037 54 Martin
Slovakia

Instructions for authors: http:||www.jfmed.uniba.sk (Acta Medica Martiniana)

Tlač:
P+M Turany

SEMI-INTERACTIVE DETECTION OF ACTION POTENTIALS USING LOCAL WAVE FEATURES AND CLUSTERING

LUKÁŠ TÛMA[1,2], KLÁRA BERNÁŠKOVÁ[2], JAN MAREŠ[2]
[1]4th Department of Internal Medicine, General Teaching Hospital in Prague, Czech Republic,
[2]Department of Normal, Pathological and Clinical Physiology, 3rd Faculty of Medicine, Charles University in Prague, Czech Republic

Abstract

Impressive achievements in neuronal waveform processing branch into abundant pool of dedicated algorithms - modifications of few core principles. This paper details design and implementation of effective customizable algorithm for detection of extracellularly recorded action potentials demonstrated on recordings from brain cortex of adult male Wistar rats. At first signal is filtered using surroundings amplitude averaging and moving average subtraction. Subsequently, location of data points in which first derivation of waveform crosses zero value steeper than in common artefacts found in background activity is estimated. For description of the point's surroundings, amplitude and steepness with their bilateral symmetry are calculated and utilized to select graphoelements that are subsequently clustered by modified K-means algorithm. This procedure allows formation of shape patterns of action potentials. Comprehensive offline analysis of our recordings demonstrated this approach as well utilizable for typifying and consecutive selection of action potentials, which fluctuate due to experimental interventions. Moreover, it facilitates distinguishing activity of separate neurons in multi-unit waveforms.

Key words: spike detection, clustering, action potential, K-means, neuronal network.

INTRODUCTION

Automated action potential detection and processing constitutes an inherent precondition for modern evaluation and interpretation of all recordings of electrical activity of excitable cells. In our experiment, changes in neuronal activity in cortex of the rat during development of cortical ischemic lesion caused by photothrombotic vessel occlusion were investigated [1, 2, 3, 4, 5]. An electric signal is recorded both from inside and from the vicinity of a developing ischemic focus. Changes in extracellular space are anticipated in our experiments. Therefore, alteration in shape and other characteristics of extracellular activity record of particular neuron can be obviously expected. This may be caused either by response of a cell (and its neighbours) to ischemia or neuron dislocation due to development of perifocal oedema as well as its eventual lysis. The nominal impedance of registration microelectrode determines the capability to register multiple unit activity. Because of mutual displacement of electrode and tissue during recording, more than one type of cell activity can be registered and the shape of action potential may vary in time.

Indisputably, the process of detection and analysis of recordings of action potentials is computationally intensive; sensitivity and specificity enhancement of an algorithm is burdened with increased computational complexity. Respective methods can be divided according to their complexity, the usage of artificial intelligence, fuzzy operators, clustering and the necessity of human interaction during the process of data evaluation. Peak tracking based on sole amplitude is an unsophisticated and simple method with linear time complexity [6, 7]. Employment of shape border constraints [8], or sliding clipping window determining steepness and amplitude of a peak has also been reported. Another method is the Maximum Integral Transform Alignment, which is based on integration of positive and negative part of an action potential [9]. Graphoelements showing little variation in time can be tracked by searching for their characteristic features [6]. Next technique that allows identifying action potentials is filtration. More sophisticated methods use transformation of a signal into another domain: for example wavelet or Fourier transforma-

Address for correspondence:
Lukas Tuma M.D. Ing., Department of Normal, Pathological and Clinical Physiology, 3rd Faculty of Medicine, Charles University in Prague, Czech Republic

tion, both otherwise profusely exploited in multimedia compression [10, 11]. Clustering of similar spikes and principal component analysis facilitate recognition of both outlying values and signals from multiple cells recorded simultaneously in one waveform – multiple unit activity [6, 7, 12]. Rapid progress in computer technology allows implementation of algorithms with growing complexity and accuracy for automated signal evaluation even in real time on machines with affordably priced computational force.

The objective of this work was to invent and implement sufficiently accurate algorithm for tracking and sorting of single unit potentials in recordings of electrical activity of one or a small group of neurons. It was necessary to create an algorithm for off-line evaluation of waveforms recorded within our experimental work. Activity changes in time as an expected response to the experimental intervention had to be considered as well. The algorithm was designed to evaluate 20 minute waveform sampled at 10 kHz in same-order time growing linearly with the number of data points.

Detection of single unit potentials is an especially specific task. Combination of previously mentioned principles is necessary to achieve high-quality results. Furthermore, additional complexity is required to evaluate recordings of varying technical quality.

METHODS

Subjects: The data were recorded in adult male Wistar rats weighing from 200 to 250 g. The animals were raised under a controlled light cycle (12 hours light, 12 hours dark, lights on at 6:00 am) with free access to food and water. All procedures were performed in accordance with Ethical Guidelines of the 3rd Faculty of Medicine, Charles University, and in agreement with Guidelines of the Animal Protection Law of the Czech Republic, which corresponds to respective EU regulations. Ethical Commission approved the experimental protocol. Special care was given to minimize animal suffering. After induction to anaesthesia (Urethane, 20%, 6.5ml/kg, intraperitoneally), the scull was uncovered and two trephine openings 3 mm in diameter were made bilaterally over the somatosensory cortex. A peripheral catheter was inserted into caudal vein and the animal was fixed in a stereotactic apparatus. Glass microelectrodes (impedance 6 × 1.5 M) filled with 3 mol NaCl solution were used for registering the activity of cortical neurons in the right sensorimotor area. Obtained signal was amplified with an operational amplifier with adjustable resistance in the feedback loop. A 16-bit A/C converter Micro 1401 by Cambridge

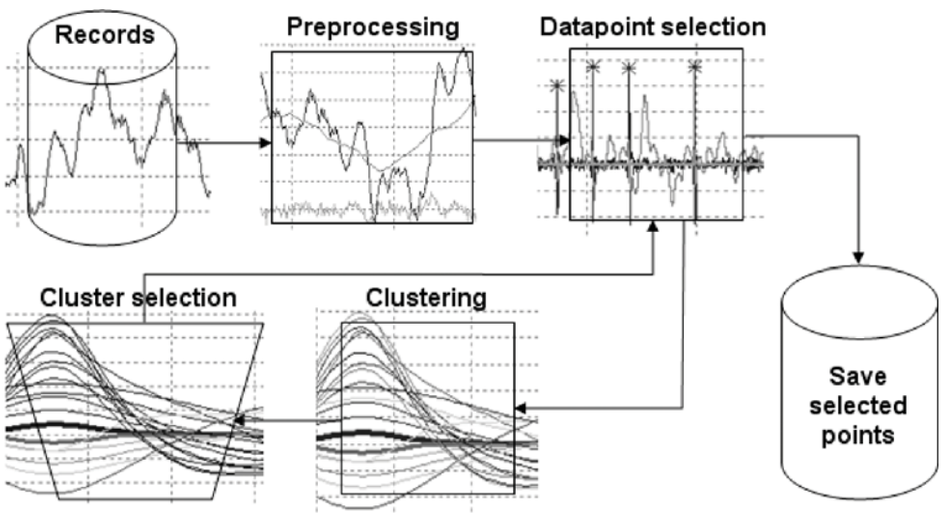


Fig. 1: Algorithm flowchart.

Electronic Design with input range ± 5 V was used for data digititalization. The experiment started with registration of 5 minutes of spontaneous waveform. Afterwards either photosensitive dye Rose Bengal (20 mg/2 ml/kg, dissolved in 0.9 % NaCl) was applied slowly into systemic circulation in the experimental group or saline solution in equal volume in the control group. Subsequently a diode laser irradiation was used as a light source for photothrombosis (duration 9 minutes, wavelength 532 nm, power density 50 mW/mm^2, illuminated area < 1 mm^2). After that, recording continued for another 11 minutes.

Algorithm: The algorithm was implemented in C++, which was chosen for its simplicity, versatility and effective code generation. The recordings were processed on a PC running on AMD Sempron 3000+ mobile processor with 512 MB of RAM. We decided to implement a three-phase waveform evaluation procedure (Fig. 1).

First phase: Initially, the waveform is preprocessed. Low frequencies are eliminated by subtraction of moving average from the waveform (Fig. 2a). Duration of the base of sliding average is at least three times longer than the expected duration of an action potential, which attains in our case typically 12 points, i.e. 1.2 ms. A 36 points long base yields cut-off frequency around 100 Hz. Waveform smoothing and filtering off a high-frequency noise is achieved by substitution of a data point with an average of its surroundings with a typical size of ± 0.1 ms i.e. ± 1 data point in our particular case (Fig. 2b).

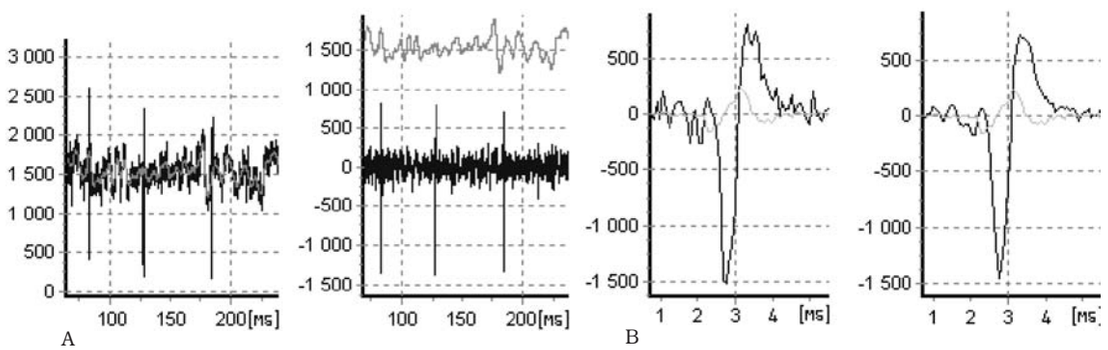


Fig. 2. A: Moving average – a highpass filter: original waveform (black) and moving average (grey) on the left; filtered signal (black) and subtracted moving average (grey) on the right.
B: Neighborhood averaging – a lowpass filter: original waveform (black) and steepness (grey) on the left, smoothed waveform (black) on the right.

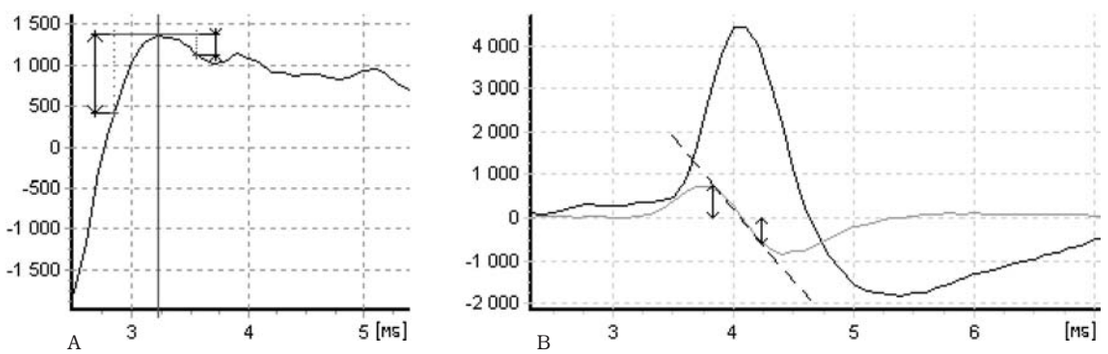


Fig. 3. A: Asymmetry of the data point neighborhood: waveform (black), arrowheads delineate amplitude variation in constant time interval that corresponds with wave asymmetry.
B: Steepness of the data point neighborhood: original waveform (black), steepness (grey). Arrowheads delineate steepness variation in constant time interval.

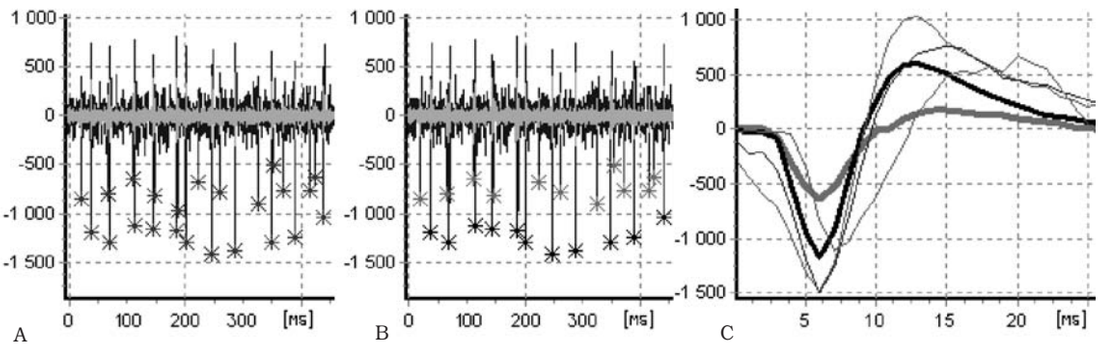


Fig. 4. A: Preselected action potentials: preprocessed waveform (black) with preselected points marked with stars (*). B: Action potentials matched to cluster patterns: preprocessed waveform (black) with preselected points marked with stars (*), the two colors distinguish two clusters.waveform (black). C: Clusters matching action potentials in Figure 4b.

Second phase: The objective of the second part of waveform processing is to reduce the number of points entering the third, the most computationally demanding part to minimum. We search the points that resemble action potentials in their features or in features of their surroundings. Points embodying defined characteristics mark most likely spikes we are looking for. Amplitude, steepness (Fig. 3a) and symmetry (Fig. 3b) were chosen as the most helpful to differentiate action potential from other activity. Both amplitude and steepness are considered as upper boundary values for the background noise and are calculated from the data point surroundings with adjustable size, typically ± 5 data points, i.e. ± 0.5 ms in our particular case. The symmetry criterion determines the maximal ratio of amplitude or steepness before and after particular data point, thus excluding usually asymmetric artefacts. Common values range from 0.1 to 10-fold. At the end of the second part, the set of „interesting“ points contains only a fraction of its initial size (Fig. 4a).

Third phase: The last part, the clustering, is operator dependent. The objective is to assign preselected waves (the output from the second part) to a defined number of the closest patterns. This allows to pinpoint the time variability of action potentials or to distinguish activity of multiple cells. For clustering, modified K-means algorithm was chosen [9, 12, 13, 14, 15, 16]. The algorithm assigns particular vectors $\boldsymbol{x}_1, \boldsymbol{x}_2 \ldots \boldsymbol{x}_m$ with n components as data points of action potentials in our case to the closest cluster pattern. The pattern, which has minimal distance from a particular vector, is updated proportionally to its strength (i.e. the number of already associated vectors), so that their mutual distance decreases. This step guarantees convergence of the algorithm as well. The distance between two vectors is defined as:

$$|| \boldsymbol{x}_1, \boldsymbol{x}_2 || = \sum_{i=1}^{n} | x_{1,i} - x_{2,i} |$$

and the closest vector is the only one with minimal distance.

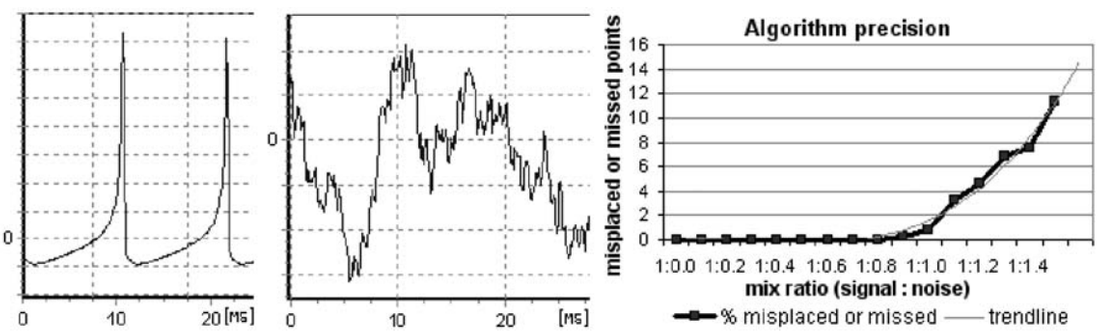
Since the diversity among the patterns can hardly be estimated in advance, the number of patterns is not implicitly dependent on the variance of the spikes, but it is preset by the operator. If the distance of two closest patterns is lower than the distance of the examined vector and its closest pattern, a new pattern originating in the particular vector is created.

If the number of patterns exceeds the limit initially set by the operator, the two closest merge proportionally to their respective strength. Further destiny of the patterns is interactively determined by the operator considering their shape and number of associated spikes, as displayed on the screen. Vectors that were associated with undesired patterns can be easily erased. Clustering and selection can be iterated and so can clusters be divided into even smaller subsets (Fig. 4b and 4c). The third part is the most computationally demanding one: the time complexity rises about linearly with the number of preselected data points and less than exponentially with the

number of patterns. The computation time can be improved by limiting the number of clusters usually to 10 to 20.

RESULTS

The developed algorithm was successfully implemented and employed in our experimental work for detection of single unit potentials in recordings counting n = 12 million data points i.e. 20 minutes sampled at 10 kHz. Specificity and sensitivity was tested on signal mixed from artifitial neuron model [17] (Fig. 5a) and uniform background noise recording (Fig. 5b) in respective ratios. The 1:1 ratio (maximal amplitude of generated signal : maximal amplitude of noise) yielded brilliant 0.89% misplaced or missed action potentials (Fig. 5c). The time complexity of the second part (the point preselection) is O(n) and of the third part (the clustering) $O(nv^2)$, where v is the preset number of patterns. The final output of the program is a binary file containing vectors of selected action potentials including their timestamp. Groups of such files are subsequently analyzed in a different environment. The program features output from intermediate stages of the analysis (patterns, selected vectors) in form of a table that can be easily processed in a standard spreadsheet program.



A B C

Fig. 5. A: Artificial neuron model; B: Background noise; C: Algorithm precision chart.

DISCUSSION

Semi-interactive detection algorithm of action potentials using local wave features and clustering combines two principles of detection of graphoelements: tracking of local features of a wave and clustering with subsequent selection of patterns. In certain parts, it is operator dependent. In our recordings, it attains sufficient accuracy and it can be efficiently employed for evaluation of non-stationary recordings of action potentials. Measured in the percentage of missed or misplaced spikes, our algorithm outperforms many other methods. However, this point of view is biased by the diversity of benchmarks used in establishing algorithm performance.

The development of waveform in time as a response to experimental intervention is the main subject of our research. Semi-interactive method for detection of action potentials allows us to observe changes in electrical activity (discharge frequency, coupling of discharges) of observed cells as a reaction to ischemia.

Neuronal death begins within 1 or 2 minutes after complete vascular occlusion in the core of the ischemic focus. Simultaneously, an area with gradually decreasing blood flow called penumbra develops around the ischemic core. Some neurons in penumbra become electrically inactive. These silent neurons may perish or recover. Our research revolves around changes in unit activity shortly before the activity disappears and in activity of surviving neurons. We assume that a change in activity of neurons synaptically connected with those in penumbra or in the core area

takes place. Therefore we evaluate unit activity of cortical neurons in contralateral hemisphere.

For routine evaluation, setup profiles for all adjustable constants will be necessary to implement in the program in order to introduce uniformity in the data processing and allow semi-skilled users to use it effectively. Our system is comparably as fast as marketed programs, however, its principal strength lies in effortless adjustability to a particular experiment.

REFERENCES

1. Pevsner PH, Eichenbaum JW, Miller DC, Pivawer G, Eichenbaum KD, Stern A, Zakian KL, Koutcher JA. A photothrombotic model of small early ischemic infarcts in the rat brain with histologic and MRI correlation. J Pharmacol Toxicol Methods 2001; 45 (3): 227-33.
2. Matejovska I, Bernaskova K, Krysl D, Mares J. Influence of melatonin pre-treatment and preconditioning by hypobaric hypoxia on the development of cortical photothrombotic ischemic lesion. Physiol Res 2007.
3. Watson BD, Dietrich WD, Busto R, Wachtel MS, Ginsberg MD. Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol 1985; 17 (5): 497-504.
4. Yao H, Sugimori H, Fukuda K, Takada J, Ooboshi H, Kitazono T, Ibayashi S, Iida M. Photothrombotic middle cerebral artery occlusion and reperfusion laser system in spontaneously hypertensive rats. Stroke 2003; 34 (11): 2716-21.
5. Markgraf CG, Kraydieh S, Prado R, Watson BD, Dietrich WD, Ginsberg MD. Comparative histopathologic consequences of photothrombotic occlusion of the distal middle cerebral artery in Sprague-Dawley and Wistar rats. Stroke 1993; 24 (2): 286-92; discussion 292-3.
6. Lewicki MS. A review of methods for spike sorting: the detection and classification of neural action potentials. Network 1998; 9 (4): R53-78.
7. Bergman H, DeLong MR. A personal computer-based spike detector and sorter: implementation and evaluation. J Neurosci Methods 1992; 41 (3): 187-97.
8. Stewart CM, Newlands SD, Perachio AA. Spike detection, characterization, and discrimination using feature analysis software written in LabVIEW. Comput Methods Programs Biomed 2004; 76 (3): 239-51.
9. Andrew D, Craig AD. Responses of spinothalamic lamina I neurons to maintained noxious mechanical stimulation in the cat. J Neurophysiol 2002; 87 (4): 1889-901.
10. Rinberg D, Bialek W, Davidowitz H, Tishby N. Spike sorting in the frequency domain with overlap detection. Physics.data-an 2003; arXiv:physics/0306056v2: (Internet: http://arxiv.org/abs/physics/0306056).
11. Hulata E, Segev R, Ben-Jacob E. A method for spike sorting and detection based on wavelet packets and Shannon's mutual information. J Neurosci Methods 2002; 117 (1): 1-12.
12. Horn CC, Friedman MI. Detection of single unit activity from the rat vagus using cluster analysis of principal components. J Neurosci Methods 2003; 122 (2): 141-7.
13. Takahashi S, Anzai Y, Sakurai Y. Automatic sorting for multi-neuronal activity recorded with tetrodes in the presence of overlapping spikes. J Neurophysiol 2003; 89 (4): 2245-58.
14. Borisoff JF, McPhail LT, Saunders JT, Birch GE, Ramer MS. Detection and classification of sensory information from acute spinal cord recordings. IEEE Trans Biomed Eng 2006; 53 (8): 1715-9.
15. Brozovic M, Andersen RA. A nonparametric quantification of neural response field structures. Neuroreport 2006; 17 (10): 963-7.
16. Takahashi S, Anzai Y, Sakurai Y. A new approach to spike sorting for multi-neuronal activities recorded with a tetrode—how ICA can be practical. Neurosci Res 2003; 46 (3): 265-72.
17. Izhikevich EM. Simple Model of Spiking Neurons. IEEE Transactions On Neural Networks 2003; 14 (6): 1569-1572.

Aknowledgements: This work was supported by following grants: VZ 0021620816, GAUK 104/2004/C/3LF.

TUMOUR SUPPRESSIVE EFFECT OF LETROZOLE IN MAMMARY CARCINOGENESIS OF FEMALE RATS

VLADIMÍRA SADLOŇOVÁ[1], PETER KUBATKA[1], IVETA ŠVECOVÁ[1], KAROL KAJO[2], GABRIELA NOSÁĽOVÁ[1], JURINA SADLOŇOVÁ[3]

[1]Department of Pharmacology, Comenius University, Jessenius Faculty of Medicine, Martin, [2]Department of Pathology, Comenius University, Jessenius Faculty of Medicine, Martin, [3]Clinic of Internal Medicine I, Comenius University, Jessenius Faculty of Medicine, Martin, Slovakia

Abstract

The aim of this study was to make experimental premenopausal model of the mammary carcinogenesis that would enable to assess preventive tumour suppressive effect of aromatase inhibitor letrozole. We also evaluated side-effects or adverse effects of letrozole on an organism. This model mimicked situation in healthy, but from the point of view of the development of breast cancer, high-risk premenopausal women.

Female Sprague-Dawley rats used in the experiment were divided into 3 groups. Aromatase inhibitor letrozole was used as a chemopreventive agent taken by the animals in the food during the whole period of time of the experiment. Group 1 - the control group had taken food without letrozole, the groups 2 and 3 with letrozole in various concentrations. To induce mammary carcinogenesis carcinogen N-methyl-N-nitrosourea (NMU) was used.

In the control letrozole-free group 75 per cent - incidence of mammary tumour was observed. In the group 2 with letrozole administered in the concentration of 1 mg per 1 kg of food the incidence of mammary tumours was 5.2 per cent. In the group 3 with letrozole concentration of 10 mg per 1 kg of food total suppression of the mammary carcinogenesis was observed.

Our experiment showed apparent preventive tumour suppressive effect of letrozole in the premenopausal model of mammary carcinogenesis in the female Sprague-Dawley rats.

Key words: mammary carcinogenesis, chemoprevention, aromatase inhibitors, letrozole, female rats

INTRODUCTION

Till recently, the most frequently used agent in the hormonal treatment of all stages of breast cancer in the women with receptor-dependent tumours was the selective estrogen receptor modulator tamoxifen, regardless of their age and menopausal condition (1). In spite of its efficacy, tamoxifen treatment was associated with many serious adverse effects. This was the reason to search for new therapeutic alternatives with similar or even higher efficacy and lower toxicity.

In postmenopausal women, production of estrogens in ovaries is suppressed. Peripheral tissues, e.g. fat tissue, muscles, liver or breast, are becoming main sources of estrogen synthesis. Due to the effects of aromatase enzyme, it catalyzes conversion of androgens, androstenedione and testosterone into estrogens, estrone and estradiol. This knowledge led to the development of agents denoted as aromatase inhibitors, which block the process of estrogen production supporting the growth of hormone-dependent breast tumours (2). At present, new aromatase inhibitors - anastrozole, letrozole and exemestane are approved and used in clinical practice. There is evidence, that new non-steroidal aromatase inhibitor letrozole, a derivate of benzyltriazole, is superior to tamoxifen in the first-line therapy for advanced breast cancer in postmenopausal women (3) and also in adjuvant therapy of postmenopausal women with breast cancer (4, 5). It was assumed that aromatase inhibitors might be more effective than tamoxifen also in neoadjuvant therapy (6). Based on the results of the above cited studies, letrozole was approved as the drug for the first-line therapy of postmenopausal women with advanced receptor-positive breast cancer, also in adjuvant and neoadjuvant therapy of postmenopausal women with a receptor-positive early-stage breast cancer.

Address for correspondence:
Vladimíra Sadloňová, MD., Department of Pharmacology, Comenius University, Jessenius Faculty of Medicine, Sklabinská 26, SK-037 53 Martin, Slovakia
Phone: +421 43 4132535, e-mail: vsadlonova@jfmed.uniba.sk

Monotherapy with aromatase inhibitors is not used in the premenopausal women with breast cancer. But in the North America approximately 22 per cent of all cases of breast cancer are diagnosed in the women younger than 50 years who are in premenopausal period (7). The efficacy and toxicity of aromatase inhibitors in the treatment of premenopausal women with breast cancer are discussed among oncologists (8, 9, 10). In our experiment we investigated preventive effects of letrozole in premenopausal mammary carcinogenesis of female rats.

METHODS

In our experiment we used 60 intact female Sprague-Dawley rats (AnLab, Prague, Czech Rep.) 33 - 37 days old, weighing 130 - 155 grams. The Sprague-Dawley rats belong to highly susceptible rat strain, which ensure high incidence and frequency of mammary tumours. The animals were divided into plexi cages (5 animals/1 cage) and for 7 days they were adapting to the standard conditions of vivarium (temperature 23 ± 2 °C, relative humidity 50 - 60 %, artificial light regimen, light : dark/12 : 12). The animals were taken standard food for rats (Kocanda Mlyn, Prague, Czech Rep.) and water ad libitum. All procedures were carried out according to EU directives and reviewed by Ethical Committee of the Comenius University.

The animals were divided into 3 groups (20 animals in 1 group). Aromatase inhibitor of letrozole was used as a chemopreventive agent administered in rat food. Control group 1 had taken the food which did not contain letrozole. Group 2 had taken the food containing letrozole in concentration of 1 mg per 1 kg of food and the group 3 the food containing letrozole in concentration of 10 mg per 1 kg of food.

The N-methyl-N-nitrosourea (NMU) as chemocarcinogen (Sigma, Deisenhofen, Germany) was used to induce mammary carcinogenesis. Immediately before its application, NMU was dissolved in saline (0.5 ml/1 animal) and then injected intraperitoneally on the 44^{th} and 51^{st} days in the dose of 50 mg/kg of the animal's body weight. Application of the NMU during period of 40 - 60 postnatal days is important because the highest sensitivity of rat mammary gland occurring at this time.

Once a week the rats were weighed and palpated. The body weight of animals was evaluated and the local mammary tumours were assessed in terms of their presence, number, place and size. In the 8^{th} and 16^{th} weeks of the experiment water and food intake was measured. At the end of the experiment, i.e. 17 weeks after the application of the first NMU dose, the animals were killed by quick decapitation. Then their blood was taken to examine its biochemical parameters to find the effect of letrozole on lipid profile - total cholesterol, high - density lipoprotein cholesterol (HDL), low - density lipoprotein cholesterol (LDL) and triacylglycerols (TAG) (Institute of the laboratory diagnostics Alpha Medical a.s. Ružomberok). During autopsy, mammary tumours, uterus, vagina and other pathologically changed tissues were excised. The samples (uterus, vagina) were weighed and together with the other samples sent for histological analysis (Department of Pathological Anatomy, Jessenius Faculty of Medicine, Comenius University in Martin).

The tumour incidence was assessed by Mann-Whitney U-test, the other parameters by one-way variance analysis (ANOVA) or Kruskal-Wallis test.

RESULTS

In our experiment a profound preventive tumour suppressive effect of letrozole in the premenopausal model of mammary carcinogenesis in female Sprague-Dawley rats was observed (Fig. 1, 2). In the control letrozole-free group 75 per cent incidence of the mammary tumours was found. In the group 2 where letrozole was administered in concentration of 1 mg per 1 kg of food, 5.2 per cent tumour incidence was observed. It means that letrozole given even in lower concentration resulted in significant decrease of incidence of the mammary tumours ($P<0.00002$) as well as in their frequency ($P<0.00002$) in comparison with untreated animals. In the group 3 where letrozole in concentration of 10 mg per 1 kg of food was administered, total suppression of mammary carcinogenesis was observed.

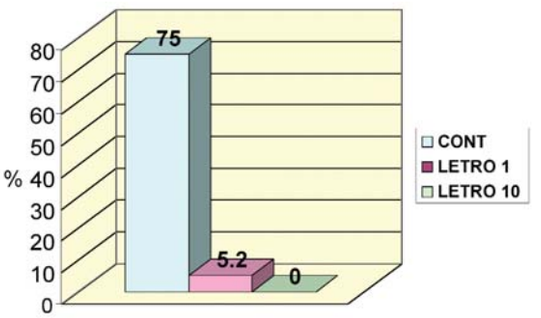


Fig. 1 Incidence of mammary tumours. CONT – control group, LETRO 1 – group with administered letrozole in concentration of 1 mg/kg in food, LETRO 10 - group with administered letrozole in concentration of 10 mg/kg in food. Data are expressed as per cents. Significantly different, CONT vs. LETRO 1 P<0. 00002.

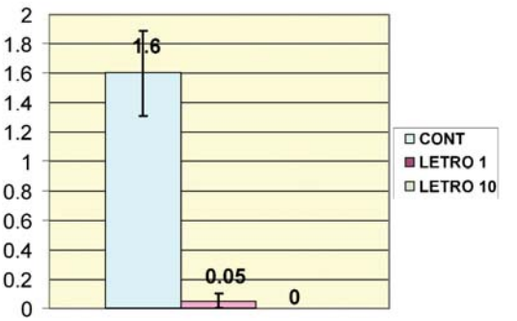


Fig. 2 Frequency of mammary tumours per group. CONT – control group, LETRO 1 – group with administered letrozole in concentration of 1 mg/kg in food, LETRO 10 - group with administered letrozole in concentration of 10 mg/kg in food. Data are expressed as meansSEM. Significantly different, CONT vs. LETRO 1 P<0. 00002.

Altogether 33 mammary tumour samples were histologically analysed. In the control letrozole-free group there were 29 cases of malignant adenocarcinomas and 2 adenomas and 1 fibroadenoma as benign tumours. In the group 2 where letrozole in concentration of 1 mg per 1 kg of food was given, 1 fibroadenoma-type tumour was found. No mammary tumours were found in the group 3 with letrozole given in concentration of 10 mg per 1 kg of food. The tumours were classified according to the criteria for classification of rat mammary tumours (11).

Some adverse effects of letrozole treatment in this experiment were observed. The atrophic changes in the endometrium of uterus and tile-shaped epithelium in the vagina were found. The degree of atrophy was in correlation with the concentration of letrozole given in food. Further, significant increase in TAG levels and body weight was observed. The body weight gain was associated with higher food intake.

DISCUSSION

Estrogens and their metabolites have been implicated in both the initiation and the progression of breast cancer. Aromatization is the major mechanism of estrogen synthesis in postmenopausal women. In these women, aromatase inhibitors block biotransformation of adrenal androgens to estrogens in peripheral tissues (including the breast, muscle, liver, and adipose), resulting in undetectable levels of plasma estrogens. For that reason, aromatase inhibitors are primarily used in the breast cancer treatment in postmenopausal population.

Application of the aromatase inhibitors in premenopausal women with breast cancer has no established role in clinical practise. It is well documented that ovaries in premenopausal women are resistant to the effects of first generation aromatase inhibitors (12). But results of some experimental works offer hypothesis that they would be suitable also for premenopausal women with breast cancer (13, 14, 15). In above cited experiments, the key role of in situ estrogen synthesis via aromatase in the mammary gland tissue in the development and progression of breast cancer was proved. It is also supposed, that estrogen synthesized in situ has greater influence on the development of breast cancer than estrogen produced by ovaries.

Our model of premenopausal mammary carcinogenesis in female Sprague-Dawley rats showed high tumour suppressive effect of letrozole. The adverse effects of letrozole observed on the lipid metabolism and genital organs were the result of the suppression of estrogen synthesis. In the future it will be also necessary to investigate the effects of letrozole on bone tissue, sex hormone levels and receptors in target tissues. It is necessary to assess the benefit/risk ratio of letrozole application in premenopausal breast cancer women.

REFERENCES

1. Early Breast Cancer Trialist's Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomized trials. Lancet 1998; 351: 1451-1467.
2. Buzdar A, Howell A. Advances in aromatase inhibition: clinical efficacy and tolerability in the treatment of breast cancer. Clin Cancer Res 2001; 7: 2620-2635.
3. Mouridsen H, Gershanovich M, Sun Y, Pérez-Carrión R, Boni C, Monnier A, Apffelstaedt J, Smith R, Sleeboom HP, Jaenicke F, Pluzanska A, Dank M, Becquart D, Bapsy PP, Salminen E, Snyder R, Chaudri-Ross H, Lang R, Wyld P, Bhatnagar A. Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: analysis of survival and updates of efficacy from the International Letrozole Breast Cancer Group. J Clin Oncol 2003; 21: 2101-2109.
4. Goss PE, Ingle JN, Martino S, Robert NJ, Muss HB, Piccart MJ, Castiglione M, Tu D, Shepherd LE, Pritchard KI, Livingston RB, Davidson NE, Norton L, Perez EA, Abrams JS, Therasse P, Palmer MJ, Pater JL. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. N Engl J Med 2003; 349: 1793-1802.
5. Thürlimann BJ, Keshaviah A, Coates AS, Mouridsen H, Mauriac L, Forbes JF, Paridaens R, Castiglione-Gertsch M, Gelber RD, Rabaglio M,Smith I, Wardley A, Price KN, Goldhirsch A. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. N Engl J Med 2005; 353: 2747-2757.
6. Dixon JM, Love CD, Renshaw L, Bellamy C, Cameron DA, Miller WR, Leonard RC. Lessons from the use of aromatase inhibitors in the neoadjuvant setting. Endocr Relat Cancer 1999; 6: 227-230
7. McPherson K, Steel CM, Dixon JM. Breast cancer – epidemiology, risk factors, and genetics. Clin Rev 2000; 321: 624-628.
8. Santen RJ, Yue W, Naftolin F, Mor G, Berstein L. The potential of aromatase inhibitors in breast cancer prevention. Endocr Rel Cancer 1999; 6: 235-243.
9. Goss PE, Strasser K. Aromatase inhibitors in the treatment and prevention of breast cancer. J Clin Oncol 2001; 19: 2767.
10. Freedman OC, Verma S, Clemons MJ. Pre-menopausal breast cancer and aromatase inhibitors: treating a new generation of women. Breast Cancer Res Treat 2006; 99: 241-247.
11. Russo J, Russo IH, Rogers AE, Van Zwieten MJ, Gusterson B, Pathology of tumours in laboratory animals. Tumours of the rat. Tumours of the mammary gland. IARC Sci Publ 1990; 99: 47-78.
12. Santen RJ, Samojlik E, Wells SA. Resistance of the ovary to blockade of aromatization with aminoglutethimide. J Clin Endocrinol Metabol 1980; 51: 473-477.
13. Bernstein LM, Larionov AA, Kyshtoobaeva AS, Pozharinsski KM, Semiglazov VF, Ivanova OA. Aromatase in breast cancer tissue localization and relationship with reproductive status of patients. J Cancer Res Clin Oncol 1996; 122: 495-498.
14. Bulun WR, Sharda G, Rink J, Sharma S, Simpson ER. Distribution of aromatase P450 transcripts and adipose fibroblasts in the human breast. J Clin Endocrinol Metabol 1996; 81: 1273-1277.
15. Yue W, Wang JP, Hamilton CJ, Demers LM, Santen RJ. In situ aromatization enhances breast tumour estradiol levels and cellular proliferation. Cancer Res 1998; 58: 927-932.

Supported by grants UK/66/2007, UK/67/2007 and ESF-Project SOP LZ 2005/NP1 – 027

AUTOIMMUNE THYROIDITIS AND ISOHORMONAL THERAPY IN CHILDHOOD – 10 YEARS FOLLOW UP

JOZEF MICHÁLEK, GEDEON FODOR, EVA MENDELOVÁ, MARIÁN ŠMOLDAS
National Pediatric Endocrinological and Diabetological Centre, National Endocrinological and Diabetological Institute, Lubochna, Slovakia

Abstract

Autoimmune thyroiditis is the most frequent thyroid disorder in the countries with sufficient iodine balance. There are two opinions about its therapy. First one, which prefers thyroxine therapy only in the period of thyroid hypofunction, while the other one starts with therapy at the time of eufunction, using low doses of thyroxine – the so called isohormonal therapy (11). The authors compared both methods of therapy during 10 years of follow up.

Key words: autoimmune thyroiditis, isohormonal therapy

INTRODUCTION

In 1912 the Japanese endocrinologist Hashimoto described four cases of thyroiditis with typical lymphoid infiltration and alternatively named this disease as chronic diffuse lymphoid thyroiditis (CDLT). For more than 60 years it was considered extremely rare, because of its asymptomatic course. However, the progress in diagnostic methods since the 1980s including ultrasonography, immunodiagnostics of antiperoxidase (TPOab) and antithyroglobulin (TGab) antibodies, fine needle biopsy (FNB) showed CLDT as a most frequent thyroid disorder in iodine replete countries (2). It was formerly supposed to be a disease of higher age, but the findings within last 20 years showed increasing incidence in childhood and adolescence (3). In Slovakia, Podoba and Hnilica (7) found CLDT in 4.2 % of 17-19- year-old girls and Podoba (8) in 5.2 % adolescent girls and 1.2 % adolescent boys. Females are more frequently diseased (8:1 – 22:1) than males and also the compound incidence in some chromosomal aberancies (such as Turner syndrome, Klinefelter syndrome, m. Down) is more frequent. CDLT is also associated in increasing rate with thyroid carcinoma, lymphoma and also with other autoimmune diseases (pernicious anaemia, systemic lupus erythematosus, rheumatoid arthritis, Addison disease, hypoparathyroidism and diabetes mellitus as well as with polyglandular autoimmune endocrine syndrome). Volpé (15) classified autoimmune thyreoiditis in five various clinical courses, but the last findings testified that these are only variants of the same disease. Lymphocytic infiltration keeps going to fibrotisation and gradual athrophy of follicles (1). This course depends on the type of produced antibodies. Thus, TPOab can be cytotoxic and thus to decrease the thyroid function, but at the same time can be positive also antibodies against TSH receptor as in Basedow disease, which can stimulate not only the goiter growth but also the hyperfunction (hashitoxicosis). TRab can also inhibit TSH binding to its receptors and thus lead to early hypothyroidism. Familial incidence is known, often in three subsequent generations, but the youngest generation is diseased hardly. Some authors found the association between CDLT and HLA-DR3, HLA- DR4 and HLA- DR5.

The course of CDLT can be affected also by some external factors (smoking, viral infections, iodine intake: iodine overtreatment can induce autoimmune answer, concurrent deficiency of iodine and selenium, endocrine disruptors : polychlorinated biphenyls, pesticides (4). The course of CDLT plays a special role during childhood, since in preschool children of mothers suffering from CDLT an average decrease of IQ by about 10 % has been found (5,6,10). Typical course of CDLT is characterized by an uprolonged subclinical phase of hypothyrodism. Such findings considerably influenced the therapeutic procedure which was based on the opinion that the treat-

Address for correspondence:
Doc. MUDr. Jozef Michálek, PhD, National Pediatric Endocrinological and Diabetological Centre, National Endocrinological and Diabetological Institute, Lubochna, Slovakia

ment by thyroxine should be started only in the period of evident hypothyrodism. Other hypothesis ("isohormonal therapy") calculate with possible interaction between low doses of tyroxine and receptors(11, 12). There are only indirect confirmations for positive effect of this type of therapy such as decreased thyroid volume, and delayed start of hypothyroidism.

PATIENTS AND METHODS

This work is based on long term follow-up of 44 children and adolescents treated in National Pediatric Endocrinological and Diabetological Centre in National Endocrinological and Diabetological Institute at Ľubochňa which were divided in two groups: 1st group (A) consisting of 32 children (29 girls and 3 boys) diagnosed as CDLT in average age of 12.9 years (range of 5.9 – 16.2 years) treated by low doses (25 – 50 Ķg) of thyroxine since 1997 until 2007 (Fig1); 2nd control group (B) was created by 12 children (10 girls and 2 boys) with average age of 13.1 years (5.1-17.7 years) diagnosed and followed at the same time and therapy was started only in time of elevation of TSH (Fig. 2). The diagnosis was established by simultaneous findings of positive TPOab or TGab (or both) antibodies and positive picture of USG examination. Function of thyroid gland was controlled twice yearly, USG findings were compared each second year. Thyroxine therapy in both groups was continual, without interruption. Girls were examined always in follicular phase of menstruation cycle.

Among the total numbers of children shown above there were some with familial incidence of CDLT, such as 11 children (8 girls and all 3 boys) in the group A (Fig. 3) and 4 children (3 girls and 1 boy) in the group B (Fig. 4). The values of TSH between 4.5 and 6.0 mU/l were considered as subclinical hypothyreosis, values over 6mU/l were considered as evident hypothyreosis. Volume of thyroid gland was compared with epidemiological norms for the particular age groups. Results of both groups were compared using Student's – test.

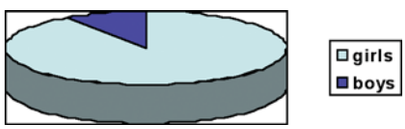


Fig.1: Gender differences group A

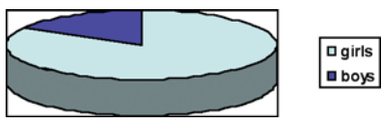


Fig.2: Gender differences - group B

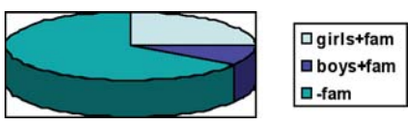


Fig.3: Familial incidence of CDLT: group A

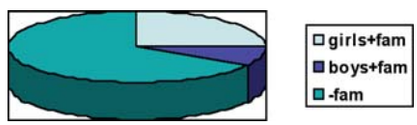


Fig.4: Familial incidence of CDLT: group B

RESULTS

In the group A the average decrease of thyroid volume against the norm for appropriate age group of age was 19.2 % (range of 13.6 – 21.8 %) at the end of the followed period (Fig.5). In contrast, in the thyroid volume in group B decreased by 10.1 % (range 6,2 – 14,1 %). There was no reduction of thyroid volume found in this group (Fig.6). Using the t-test we found significantly higher ($p < 0.01$) decrease of thyroid volume in group A. Thyroid function in the group A (except two cases of latent – subclinical – hypothyroidism) which needed substitution by thyroxine, all other patients were euthyreotic (Fig.7). In the group B, however, 8 patients developed overt clinical hypothyroidism in average at 6 years after the diagnosis of the disease (Fig.8). In two cases the progression to hyperfunctional hashitoxicosis was observed, which needed surgical treatment.

Fig. 5: Reduction of thyroid volume: Group A

Fig. 7: Subclinical hypothyreosis: Group A

Fig. 8: Evident hypothyreosis: Group B

DISCUSSION

Changes of thyroid volume should be considered only as indirect signs of improving or worsening of the disease. Early thyroid atrophy with a significant decrease of volume shows a typical ultrasonographic image (13,14). This is the only situation when the decrease of volume shows worsening of the disease. All other cases of thyroid volume reductions indicate, according to the author's opinion, improving of the disease.

Still more evident are the changes of thyroid structure. Thus, homogenous structure which further changes to spotted structure and folloving signs of microfibrotisation shows worsening of the clinical picture. Changes of thyroid function are late or final signs of worsening and in childhood they are only of low significance (9). Therefore, positive changes in volumetry and persistent euthyroidism are in the author's opinion sufficient evidence for isohormonal therapy. Costs of therapy are low and the therapy using low doses of thyroxine is also very safe. Low doses probably stabilize intracellular metabolism and cellular membrane against autoimmune damage. The estimation of anti-TPO and anti-TG (TPOab, TGab) antibodies is of significance only for the assessment of diagnosis. Changes of antibodies do not correlate with the clinical picture and, therefore, in a case of positive result, a single estimation is satisfacory. The multiplication of familial disposition for autoimmune thyreoiditis is a sufficient argument for isohormonal therapy, especially in childhood. Also the stabilisation of menstrual cycle, which is often irregular in untreated girls, could be of important value for the next life. Therefore, the isohormonal therapy of CDLT improves the quality of life in this group of patients.

REFERENCES

1. Bretz JD, Baker Jr.JR. Apoptosis and autoimunne thyroid disease: folloving a trail to thyroid destruction? Clin Endocrinol 2001; 55: 1-11.
2. Dayan CM, Daniels GH. Chronic autoimunne thyroiditis. N Engl J Med 1996; 333: 99-107.
3. Dusault JH. The ancedental history of screening for congenital hypothyreoidism. J Clin Endocrinol Metab 1999; 84: 4332-3.
4. Gärtner R, Gasnier BC, Dietrich JW. Selenium suplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations. J Clin Endocrinol Metab 2002; 87: 1687- 1691.
5. Holm LE, Blomgren H, Loewhagen T. Cancer risks in patients with chronic lymphocytic thyroiditis. N Engl J Med 1985; 312: 601-604.
6. Morreale de Escobar G, Obregón MJ, Escobar del Rey F. Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyrorinemia? J Clin Endocrinol Metab 2000; 85: 3975- 3987.
7. Podoba J, Hnilica P. Thyroid volume goitre and diffuse lyphoid thyroiditis in adolescents after long-term iodine prophylaxis in Slovakia. J Endocr Invest 1992; 15: 14-15.
8. Podoba J Jr. Súčasný pohľad na problematiku ochorení štítnej žľazy na Slovensku. Recipe 1999; 6: 72-77.
9. Pop VJ, deVries E, van Baar AL. Maternal thyroid peroxidase antibodies during pregnancy: a marker of impaired child development? J Clin Endorinol Metab 1995; 80: 3561- 3566.
10. Schaaf L, Pohl T, Schmidt R. Screening for thyroid disorders in working population. Clin Invest 1993; 71: 126- 131.
11. Schloot N, Eisenbarth GS. Isohormonal therapy of endocrine autoimmunity. Immunol Today, 1995 June;16(6): 289-94.
12. Slatosky J, Shipton B, Wahba H. Thyroiditis : Differential diagnosis and management. Am Fam Physician 2000; 61: 1047- 1052.
13. Tajtáková M, Langer P, Fodor G. Epidemiologický profil objemu štítnej žľazy a tyreopatií na Slovensku. Vnitř Lék 2000; 46: 756- 761.
14. Tonchera M. TSH receptor and disease. Clin Endocrinology 1996; 44: 621- 633.
15. Volpé R.: Immunoregulation in a autoimmune thyroid disease. Thyroid 1994 Fall 4(3):373-7.

THE EPIDEMIOLOGY OF FOOD ATOPY PATCH TESTS: A STUDY OF 335 UNSELECTED SCHOOL CHILDREN AGED 10 FROM TWO EUROPEAN COUNTRIES

ROBERTO RONCHETTI[1], MILOŠ JESEŇÁK[1,2], MARIO BARRETO[1], DAGMAR TRUBAČOVÁ[1,3], ZUZANA RENNEROVÁ[1,3], VLADIMÍR POHANKA[3], PETER BÁNOVČIN[2], MARIA PIA VILLA[1]

[1]Department of Paediatrics, Second School of Medicine, University "La Sapienza", Rome, Italy
[2] Department of Paediatrics, Jessenius School of Medicine, Comenius University in Bratislava, Martin, Slovak Republic
[3] Srobar's Institute for Respiratory Diseases and Tuberculosis for Children, Dolný Smokovec, Vysoké Tatry, Slovak Republic

Abstract

Objective: Atopy patch test (APT) is a consensus procedure for diagnosing food allergy, especially in children with atopic dermatitis. The prevalence of positive APT reactions which can be expected to be found in unselected populations of adults or children is largely unknown. Nor is it clear whether positive APT reactions are more frequent in atopic patients and whether APT results are influenced by factors such as sex, geographical settings and life-style.

Study Design and Setting: In an unselected population of 335 schoolchildren aged 10 years from two European countries with some differences life style (Italy and Slovakia) we investigated the prevalence of positive ATP reactions after cow milk, hen egg, tomato and wheat flour. We also assessed a possible link between positive APT results and other laboratory characteristics of atopy (allergen skin prick tests, histamine skin reactivity and eosinophil cell counts).

Results: Atopy patch tests for foods were each positive in 5-15 % of the tested subjects with about 21 % having at least one positive APT result: in general, they were more frequently positive in Italian children. In both countries the prevalence of positive APT reactions was significantly higher in males than in females. No relationship was found between positive ATP results and histamine skin reactivity, skin prick tests for the tested food allergens or for inhalant allergens and eosinophil cell counts.

Conclusion: The findings from this study in unselected children suggest that APTs frequently elicit positive responses in unselected children populations, especially in boys, with different frequencies for particular food allergen in different geographical settings independently from the presence of positive SPT reactions.

Keywords: Atopy, atopy patch test, children, epidemiology, skin prick test.

INTRODUCTION

The "atopy patch test" (APT), defined as "a skin reaction induced in patients with atopic dermatitis (AD) by applying food allergens or aeroallergens on "non-lesional" skin", was first described in 1982 [1]. Thereafter it was used in many studies in adults and children with a diagnostic purpose similar to the one of the patches applied by dermatologists using an array of chemical substances on the skin of patients with contact dermatitis. Several technical procedures aimed to increase the permeability of the tested skin (including abrasion, stripping, and high concentrations of allergens vehiculated in special solvents), were initially widely used to facilitate positive test results [2-4]. All these procedures were later abandoned, because they proved unessential and difficult to standardize. The current standard APT method entails the use of commercial allergens or naive food allergens, Finn chambers, an occlusion time of 48 hours and reading at 48 and 72 hours (Figure 1) [5]. APT is now a test with recognised diagnostic properties, especially in children, to the diagnosis of food allergy [6-11]. From the epidemiological point of view, however, several points remain unresolved. First we are still linked to the original idea that a positive APT is an "eczematous" reaction occurring in the skin of patients with eczema, mainly because in these persons the skin was more "reactive" (or with altered permeability): the corollarium of this statement is the concept that an APT will invariably be negative

Address for correspondence:
Milos Jesenak, M.D., Ph.D.
Department of Paediatrics,Jessenius Faculty of Medicine,Comenius University
Kollarova 2, 036 59 Martin, Slovak Republic
Tel.:+421-4203-254, Fax:+421-043-422-26-78, e-mail:jesenak@gmail.com

in healthy patients [9,12-14]. This statement has never been clearly addressed or experimentally contradicted: vice versa, several reports based on small numbers of "controls" or allergic patients without eczema demonstrated a considerable percentage of positive APTs in non-eczematous patients [12,15]. More generally, the expected prevalence of positive APT reactions for different allergens in unselected populations, adults or children, is largely unknown. Another area of uncertainty is the relationship of APTs with IgE sensitization (atopy). Although an IgE-linked mechanism probably intervenes in the pathogenesis of the skin reaction of the APT, especially in the early phases of skin exposure [16-19], it is not clear whether in general an APT test is likely to be more positive in subjects with a positive skin prick test for that allergen or, in general, in atopic patients [20,21]. Although this item has been addressed in several papers, the limited number of subjects [16,21,22], mainly patients with AD [7,8,10,23-25], or other clinical conditions [11,12,16,18,26,27] precluded conclusions generally valid. These and other epidemiological characteristics of this diagnostic procedure (e.g. whether sex or geographical settings are relevant for positivity) should be addressed by large epidemiological studies in unselected populations: we think this information should precede the application of APT in the clinical field and the measure of its clinical sensitivity, specificity or positive and negative predictive value.

We designed this epidemiological study to assess the prevalence of positive food APTs and to evaluate the link between positive APT reactions and the characteristics of atopy in unselected populations of school children, not taking into consideration, in this phase, the clinical characteristics of the subjects. We performed APTs in 335 schoolchildren aged 10 years from Italy and Slovakia.

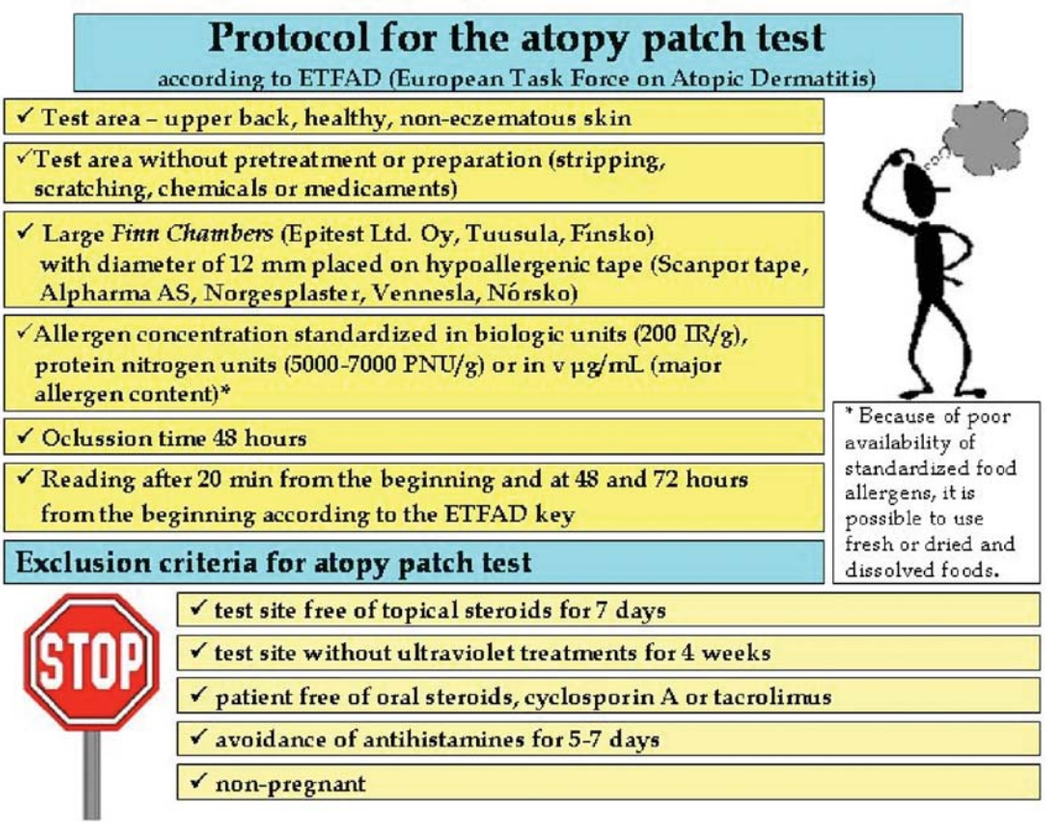


Fig.1. Protocol for the atopy patch test according to ETFAD.

MATERIAL AND METHODS

Study populations

Our study populations comprised all four-graders children attending schools in four small semi-rural towns in northern Slovakia (Poprad and Dolny Smokovec), and central Italy (Ronciglione and Caprarola). All studies were conducted between October 2002 and February 2003. We initially included in the study 439 unselected schoolchildren (234 in Slovakia and 205 in Italy). Of these 439, 104 children declined to participate or were absent on the relevant occasions or refused to undergo some programmed procedures. We report the data of 335 children, mean age 10 years, 76.3 % of those originally selected: 185 from Slovakia and 150 from Italy. The study was approved by the Ethical Committee of the Paediatric Clinic of Rome University 'La Sapienza'.

Skin testing

For one week before testing all children were asked to refrain from antihistamine medications and from inhaled or oral corticosteroids. When tested all participants denied the use of long-acting antihistamine preparations.

Atopy patch tests (APT)

APTs were done in all children with plastic quadratic chambers of diameter 10 mm (Finn Chambers, Haye's, the Netherlands) by applying one drop (50 μL) of four fresh food allergens: cow milk (containing 3.5% fat), tomato, whisked hen egg (white of egg and yolk) and wheat flour (dissolved in saline, 1g/10 mL). Food allergens were placed in the chamber and chambers were attached to an area of unaffected skin on the children's backs. As a control, physiologic solution was applied on the opposite side of the child's back. The occlusion time was 48 hours. The results were read 20 min after the chambers were removed and at 72 hours for the final test evaluation. The reading criteria were according to revised European Task Force on Atopic Dermati-

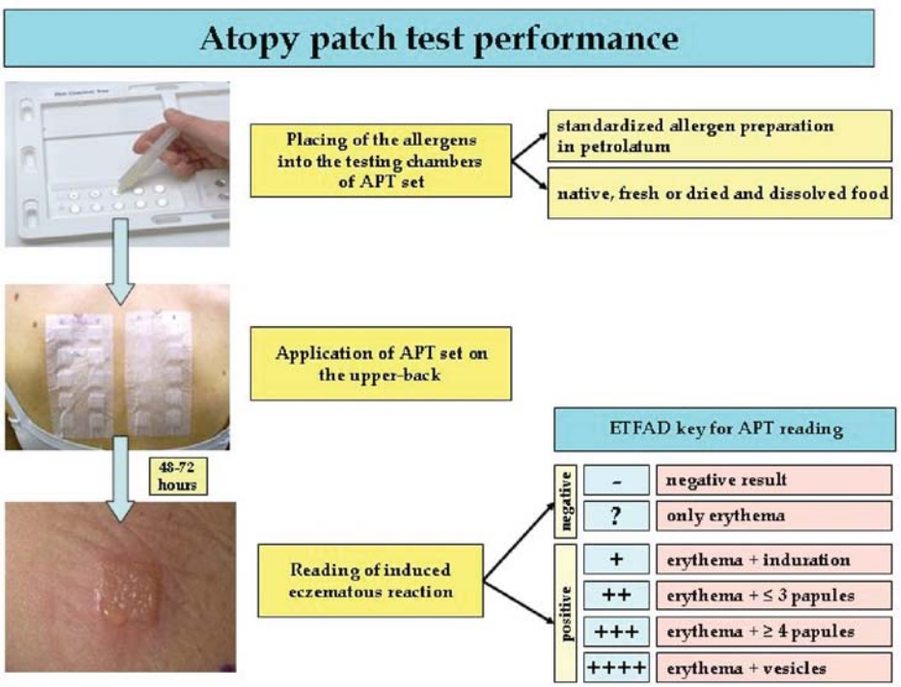


Fig.2. Atopy patch test performance.

tis key for atopy patch test reading (Figure 2) [5]. Reactions were classified as positive if there was erythema together with infiltration or papules. Erythema without palpable infiltration was considered as questionable, finally negative reaction. In both countries, all tests were done by the same two well-trained operators.

Skin prick tests (SPT)

SPT with two different concentrations of histamine were done in the left forearm: histamine 10 mg/mL on the inner side 3 cm distally from the angular bend of the elbow and histamine 1 mg/mL on the outer side 4 cm distally from the elbow bend. SPTs were done in a predefined area on the volar side of the left forearm, with a space of at least 2.5 cm between each prick. According to the International Study of Asthma and Allergic Diseases in Childhood (ISAAC) protocol phase 2 the following inhalant allergens panel were tested: *Dermatophagoides pteronyssinus, Dermatophagoides farinae*, cat dander, *Alternaria tenuis*, mixed grasses 1 (*Avena sativa, Hordeum vulgare, Secale cereale, Triticum sativum*), mixed grasses 2 (*Dactylis glomerata, Festuca pratensis, Lolium perenne, Phleum pratense, Poa pratensis*), and mixed trees (*Betula verrucosa, Corylus avellana, Alnus glutinosa*) (ALK-ABELLO, Hørsholm, Denmark). On the right volar forearm we pricked the same four fresh food allergens used for APT (cow milk, hen egg, tomato and wheat flour dissolved in 1 mg/10 ml saline). Negative controls, 50% glycerine in saline, were pricked on the left forearm. In both countries, we used the same type of histamine, allergens, negative control and 1-mm tip metallic lancets (ALK-ABELLO, Hørsholm, Denmark). The lancet was pricked vertically into the skin through each drop for 2 s with firm pressure, starting with histamine 10 mg/mL. A new lancet was used for each prick test. Ten minutes after the procedure ended, the wheals were outlined with a thin felt-tip pen. The contours were transferred to the record sheet with translucent tape. The size of each wheal was measured as the mean of the longest diameter and the diameter perpendicular to it at its mid point. A positive skin prick test was defined as a wheal ≥ 3 mm in its longest dimension. In both countries, all tests were done by the same two well-trained operators.

Serum studies

Peripheral venous blood samples were taken from all children. Eosinophils were measured within 2 hours by an automated blood cell counter (Advia 120, Bayer®, Leverkusen, Germany; Digicel 1200, Abbot®).

Statistical methods

All data are expressed as means ± *SD.* Data were analysed by the software program SPSS version 9.0 (SPSS Inc. Chicago, IL, USA). Student's two-tailed t test, chi square (χ^2) test and Fisher's exact test were used for statistical comparison. *P* values less than or equal to 0.05 were considered to indicate statistical significance.

RESULTS

Table 1 shows that studied children, when subdivided according to the nationality, present higher histamine skin reactivity, prevalence of positive skin prick tests for food or for inhalants in Italian children whereas eosinophil cell count is higher in Slovakian children. Positive skin tests (especially multiple or more intense positivity) and eosinophil cell count were found to be significantly higher in males while histamine skin reactivity did not differ between sexes.

Table 2 shows that atopy patch tests for foods were each positive in 5-15 % of the tested subjects with about 21 % having at least one positive APT result: in general, they were more frequently positive in Italian children, especially so for what cow milk and tomato were concerned. Also some differences in the prevalence of positive APT have been found between males and females: there was a general tendency for APT to be strongly positive (many papules) more frequently in males significantly so for hen egg ($p = 0.002$) and in the number of children for at least

Tab. 1. Studied populations.

	All	Italy	Slovakia	p	Males	Females	p
Number of children (males %)	335 (46%)	150 (40.7%)	185 (50.3%)	n.s.	154	181	-
Age ± SD [years]	10.10 ± 0.63	10.09 ± 0.55	10.11 ± 0.68	n.s.	10.09 ± 0.60	10.11 ± 0.66	n.s.
Histamine skin reactivity (10 mg/mL wheal diameter, mean ± SD)[mm]	4.83 ± 1.37	5.13 ± 1.43	4.59 ± 1.28	**0.001**	4.87 ± 1.44	4.79 ± 1.32	n.s.
Positive skin prick test (SPT) (wheal diameter ≥ 3 mm) [%]							
Dermatophagoides pteronyssinus	42 (13.2%)	26 (18.2%)	16 (9.1%)	**0.026**	25 (17.1%)	17 (9.8%)	n.s.
Dermatophagoides farinae	45 (14.1%)	30 (21%)	15 (8.5%)	**0.003**	26 (17.8%)	19 (11%)	n.s.
Cat dander	17 (5.3%)	10 (7%)	7 (4%)	n.s.	7 (4.8%)	10 (5.8%)	n.s.
Alternaria tenuis	21 (6.6%)	14 (9.8%)	7 (4%)	n.s.	17 (11.6%)	4 (2.3%)	**0.007**
Mixed grasses 1	50 (15.7%)	21 (14.7%)	29 (16.5%)	n.s.	32 (21.9%)	18 (10.4%)	**0.008**
Mixed grasses 2	47 (14.7%)	21 (14.7%)	26 (14.8%)	n.s.	29 (19.9%)	18 (10.4%)	**0.027**
Mixed trees	10 (3.1%)	7 (4.9%)	3 (1.7%)	n.s.	5 (3.4%)	5 (2.9%)	n.s.
Cow milk	4 (1.3%)	2 (1.4%)	2 (1.1%)	n.s.	2 (1.4%)	2 (1.2%)	n.s.
Tomato	21 (6.6%)	15 (10.5%)	6 (3.4%)	**0.021**	13 (8.9%)	8 (4.6%)	n.s.
Hen egg	2 (0.6%)	0	2 (1.1%)	n.s.	2 (1.4%)	0	n.s.
Wheat flour	16 (5%)	9 (6.3%)	7 (4%)	n.s.	10 (6.8%)	6 (3.5%)	n.s.
At least one positive SPT	104 (32.6%)	56 (39.2%)	48 (27.3%)	**0.033**	57 (39%)	47 (27.2%)	**0.033**
At least two positive SPT	76 (23.8%)	39 (27.3%)	37 (21%)	n.s.	47 (32.2%)	29 (16.8%)	**0.002**
Number of positive SPT per person [mean ± SD]	0.86 ± 1.51	1.08 ± 1.71	0.68 ± 1.30	**0.021**	1.15 ± 1.71	0.62 ± 1.27	**0.002**
Skin prick test index ± SD [mm]	5.23 ± 8.85	6.38 ± 9.64	4.30 ± 8.07	**0.040**	6.85 ± 10.10	3.87 ± 7.41	**0.003**
Eosinophil cell count (eosinophil cells/white cells) [%]	3.85 ± 2.94	2.94 ± 2.78	4.47 ± 2.88	**<0.001**	4.24 ± 3.02	3.51 ± 2.82	**0.034**

Tab. 2. Frequencies of skin results in atopy patch testing in the subgroups according to the nationality and gender.

Prevalence of atopy patch test (APT) results with four fresh food allergens							
	All	**Italy**	**Slovakia**	**p**	**Males**	**Females**	**p**
Number of children (males %)	335 (46%)	150 (40.7%)	185 (50.3%)	n.s.	154	181	-
Age ± SD [years]	10.10 ± 0.63	10.09 ± 0.55	10.11 ± 0.68	n.s.	10.09 ± 0.60	10.11 ± 0.66	n.s.
Histamine skin reactivity (10 mg/mL wheal diameter, mean ± SD)[mm]	4.83 ± 1.37	5.13 ± 1.43	4.59 ± 1.28	**0.001**	4.87 ± 1.44	4.79 ± 1.32	n.s.
Positive skin prick test (SPT) (wheal diameter ≥ 3 mm) [%]							
Dermatophagoides pteronyssinus	42 (13.2%)	26 (18.2%)	16 (9.1%)	**0.026**	25 (17.1%)	17 (9.8%)	n.s.
Dermatophagoides farinae	45 (14.1%)	30 (21%)	15 (8.5%)	**0.003**	26 (17.8%)	19 (11%)	n.s.
Cat dander	17 (5.3%)	10 (7%)	7 (4%)	n.s.	7 (4.8%)	10 (5.8%)	n.s.
Alternaria tenuis	21 (6.6%)	14 (9.8%)	7 (4%)	n.s.	17 (11.6%)	4 (2.3%)	**0.007**
Mixed grasses 1	50 (15.7%)	21 (14.7%)	29 (16.5%)	n.s.	32 (21.9%)	18 (10.4%)	**0.008**
Mixed grasses 2	47 (14.7%)	21 (14.7%)	26 (14.8%)	n.s.	29 (19.9%)	18 (10.4%)	**0.027**
Mixed trees	10 (3.1%)	7 (4.9%)	3 (1.7%)	n.s.	5 (3.4%)	5 (2.9%)	n.s.
Cow milk	4 (1.3%)	2 (1.4%)	2 (1.1%)	n.s.	2 (1.4%)	2 (1.2%)	n.s.
Tomato	21 (6.6%)	15 (10.5%)	6 (3.4%)	**0.021**	13 (8.9%)	8 (4.6%)	n.s.
Hen egg	2 (0.6%)	0	2 (1.1%)	n.s.	2 (1.4%)	0	n.s.
Wheat flour	16 (5%)	9 (6.3%)	7 (4%)	n.s.	10 (6.8%)	6 (3.5%)	n.s.
At least one positive SPT	104 (32.6%)	56 (39.2%)	48 (27.3%)	**0.033**	57 (39%)	47 (27.2%)	**0.033**
At least two positive SPT	76 (23.8%)	39 (27.3%)	37 (21%)	n.s.	47 (32.2%)	29 (16.8%)	**0.002**
Number of positive SPT per person [mean ± SD]	0.86 ± 1.51	1.08 Ī 1.71	0.68 Ī 1.30	**0.021**	1.15 Ī 1.71	0.62 Ī 1.27	**0.002**
Skin prick test index ± SD [mm]	5.23 ± 8.85	6.38 ± 9.64	4.30 ± 8.07	**0.040**	6.85 ± 10.10	.87 ± 7.41	**0.003**
Eosinophil cell count (eosinophil cells/white cells) [%]	3.85 ± 2.94	2.94 ± 2.78	4.47 ± 2.88	**<0.001**	4.24 ± 3.02	3.51 ± 2.82	**0.034**

MAKETA 7-2 po 8.1.2008 7:10 Str. 22

Tab. 2. Frequencies of skin results in atopy patch testing in the subgroups according to the nationality and gender.

	Prevalence of atopy patch test (APT) results with four fresh food allergens						
	All (335)	**Italy (150)**	**Slovakia (185)**	**p**	**Males (154)**	**Females (181)**	**p**
Atopy patch test with cow milk							
①No reaction	289 (86.3%)	128 (85.2%)	161 (87%)	n.s.	124 (80.5%)	165 (91.2%)	**0.008**
②Erythema	28 (8.4%)	8 (5.4%)	20 (10.8%)	n.s.	19 (12.4%)	9 (5%)	**0.026**
Negative results (①+②)	317 (94.6%)	136 (90.6%)	181 (97.8%)	**0.008**	143 (92.9%)	174 (96.1%)	n.s.
③Few papules (≤2)	14 (4.2%)	13 (8.7%)	1 (0.5%)	**<0.001**	9 (5.9%)	5 (2.8%)	n.s.
④Many papules (≥ 3)	4 (1.2%)	1 (0.7%)	3 (1.6%)	n.s.	2 (1.3%)	2 (1.1%)	n.s.
Positive results (③+④)	18 (5.4%)	14 (9.4%)	4 (2.2%)	**0.008**	11 (7.1%)	7 (3.9%)	n.s.
Atopy patch test with tomato							
①No reaction	302 (90.1%)	133 (88.7%)	169 (91.4%)	n.s.	131 (85.1%)	171 (94.5%)	**0.007**
②Erythema	13 (3.9%)	3 (2%)	10 (5.4%)	n.s.	11 (7.2%)	2 (2.1%)	**0.011**
Negative results (①+②)	315 (94%)	136 (90.7%)	179 (96.8%)	**0.035**	142 (92.2%)	173 (95.6%)	n.s.
③Few papules (≤2)	9 (2.7%)	6 (4%)	3 (1.6%)	n.s.	6 (3.9%)	3 (1.7%)	n.s.
④Many papules (≥ 3)	11 (3.3%)	8 (5.3%)	3 (1.6%)	n.s.	6 (3.9%)	5 (2.8%)	n.s.
Positive results (③+④)	20 (6%)	14 (9.3%)	6 (3.2%)	**0.035**	12 (7.8%)	8 (4.4%)	n.s.
Atopy patch test with hen egg							
①No reaction	261 (77.9%)	118 (78.7%)	143 (77.3%)	n.s.	105 (68.2%)	156 (86.2%)	**<0.001**
②Erythema	28 (8.4%)	9 (6%)	19 (10.3%)	n.s.	19 (12.3%)	9 (5%)	**0.026**
Negative results (①+②)	289 (82.3%)	127 (84.7%)	162 (87.6%)	n.s.	124 (80.5%)	165 (91.1%)	**0.008**
③Few papules (≤2)	22 (6.6%)	10 (6.7%)	12 (6.5%)	n.s.	11 (7.1%)	11 (6.1%)	n.s.
④Many papules (≥ 3)	24 (7.2%)	13 (8.7%)	11 (5.9%)	n.s.	19 (12.3%)	5 (2.8%)	**0.002**
Positive results (③+④)	46 (13.7%)	23 (15.3%)	23 (12.4%)	n.s.	30 (19.5%)	16 (8.9%)	**0.008**
Atopy patch test with wheat flour							
①No reaction	267 (79.7%)	115 (76.7%)	152 (82.2%)	n.s.	114 (74%)	153 (84.5%)	**0.025**
②Erythema	32 (9.6%)	15 (10%)	17 (9.2%)	n.s.	20 (13%)	12 (6.6%)	n.s.
Negative results (①+②)	309 (92.2%)	130 (86.7%)	169 (91.4%)	n.s.	134 (87%)	165 (91.2%)	n.s.
③Few papules (≤2)	24 (7.2%)	14 (9.3%)	10 (5.4%)	n.s.	14 (9.1%)	10 (5.5%)	n.s.
④Many papules (≥ 3)	12 (3.6%)	6 (4%)	6 (3.2%)	n.s.	6 (3.9%)	6 (3.3%)	n.s.
Positive results (③+④)	36 (10.8%)	20 (13.3%)	16 (8.6%)	n.s.	20 (13%)	16 (8.8%)	n.s.
Cumulative results of atopy patch test with food allergens							
All APT with no reaction ①	213 (63.6%)	94 (62.6%)	120 (64.9%)	n.s.	82 (53.2%)	131 (72.4%)	**<0.001**
At least one APT with erythema ②	65 (19.4%)	21 (14%)	44 (23.8%)	**0.035**	43 (27.9%)	26 (14.4%)	**0.003**
All APT negative (①+②)	265 (79.1%)	109 (72.7%)	156 (84.3%)	**0.013**	116 (75.3%)	149 (82.3%)	n.s.
At least one APT with few papules ③	47 (14%)	29 (19.3%)	18 (9.7%)	**0.018**	25 (16.2%)	22 (12.1%)	n.s.
At least one APT with many papules ④	38 (11.3%)	20 (13.3%)	18 (9.7%)	n.s.	24 (15.6%)	14 (7.7%)	**0.037**
At least one positive result (③+④)	70 (20.9%)	41 (27.3%)	29 (15.7%)	**0.013**	38 (24.7%)	32 (17.7%)	n.s.

one positive APT with many papules (p = 0.037). Moreover there was a consistent tendency for males to have an increased prevalence of APT eliciting simple erythema (p significant for cow milk, tomato, hen egg and for at least one APT with erythema). In females, vice versa, significantly more frequent was the finding of "no reaction at all" for all the allergens separately or considered all together. Should the erythema be considered a criterion of APT positivity the differ-

Tab. 3. Laboratory and clinical differences between subjects with negative or positive APT.

	At least one positive APT result	All APT results negative	p
Age ± SD [years]	10.10 ± 0.55	10.11 ± 0.65	n.s.
Males [%]	38 (54.3%)	116 (43.8%)	n.s.
Histamine skin reactivity (10 mg/mL wheal diameter, mean ± SD)[mm]	4.9 ± 1.22	4.81 ± 1.41	n.s.
Positive skin prick test (SPT) [%]			
Dermatophagoides pteronyssinus	13 (19.1%)	29 (11.6%)	n.s.
Dermatophagoides farinae	12 (17.6%)	33 (13.1%)	n.s.
Cat dander	1 (1.5%)	16 (6.4%)	n.s.
Alternaria tenuis	7 (10.3%)	14 (5.6%)	n.s.
Mixed grasses 1	13 (19.1%)	37 (14.7%)	n.s.
Mixed grasses 2	12 (17.6%)	35 (13.9%)	n.s.
Mixed trees	2 (2.9%)	8 (3.2%)	n.s.
Cow milk	1 (1.5%)	3 (1.2%)	n.s.
Tomato	5 (7.4%)	16 (6.4%)	n.s.
Hen egg	1 (1.4%)	1 (0.4%)	n.s.
Wheat flour	5 (7.4%)	11 (4.4%)	n.s.
At least 1 positive SPT (wheal diameter ≥ 3 mm) [%]	26 (38.2%)	78 (31.1%)	n.s.
Number of positive SPT per person [mean ± SD]	1.06 ± 1.63	0.81 ± 1.47	n.s.
Skin prick test index ± SD [mm]	6.40 ± 10.14	4.92 ± 8.47	n.s.
Eosinophil cell count (eosinophil cells/white cells) [%]	3.69 ± 2.58	3.90 ± 3.03	n.s.

Tab. 4. Relationships between the results of skin prick tests and atopy patch tests with the same four food allergens.

Concordance between the results of atopy patch test (APT) and skin prick test (SPT) for food allergens				
		APT with cow milk		
		Positive	*Negative*	p
SPT with	*Positive*	1 (0.3%)	3 (0.9%)	
cow milk	*Negative*	15 (4.7%)	299/319 (94%)	n.s.
		APT with tomato		
		Positive	*Negative*	p
APT with	*Positive*	0	21 (6.6%)	
tomato	*Negative*	19 (6%)	279/319 (87.5%)	n.s.
		APT with hen egg		
		Positive	*Negative*	p
SPT with	*Positive*	1 (0.3%)	1 (0.3%)	
hen egg	*Negative*	45 (14.1%)	272/319 (85.3%)	n.s.
		APT with wheat flour		
		Positive	*Negative*	p
SPT with	*Positive*	3 (0.9%)	13 (4.1%)	
wheat flour	*Negative*	32 (10%)	271/319 (85%)	n.s.

ence in positive APT would result much more significant for the allergens considered separately or all together ($p < 0.001$ to 0.008).

Subjects positive for APT performed with whatever allergen did not differ by sex, histamine skin reactivity, prevalence of positive SPT (foods or inhalants) or eosinophil cell count. Table 3 reports this lack of association for children with or without at least one positive APT.

Similarly, no correspondence was found between the results of APT for single food allergens and SPT performed with the same allergen (Table 4): i.e. of the 116 positive APT with any of the four foods, in only 4 cases were also positive SPT.

DISCUSSION

In our unselected population of school children subjects from two different European countries and males compared to females differed in many of the measured laboratory characteristics (histamine skin reactivity, SPT positivity, eosinophil cell counts). The prevalence of positive APT reactions to food furnished results in the same direction: higher prevalence of positive tests in Italian children and in males. The latter is a new and previously unreported finding.

Our study suggests that a positive test is to be expected in about 20 % of an unselected paediatric population (about 25 % in males and 18 % in females): different results can be expected in different geographical settings.

In our data APT results were not associated with skin reactivity to histamine. Nor were positive APT in either population associated with the positive skin prick tests performed with the same food allergens or with eosinophil cell count.

These results allow three issues for consideration. First, the significant number of subjects of the general population positive to atopy patch tests performed with four fresh foods obscures the hypothesis that APT elicits an "eczematous reaction" [9,12-14] or it is a reaction inducible only in subjects with atopic dermatitis. It appears that this skin reaction is the result of a certain type of sensitization induced by food allergens in the subjects with or without eczema (unpublished data concerning population of the same two geographical settings the prevalence of "current" eczema ranged between 5-7 %). Second, the fact that Italian and male children have higher prevalence of positive APT suggests that both environmental and genetic factors are relevant in the findings concerning this parameter: our results could have been different in other places, other populations or in different times and maybe in different ages. Third, we found a lack of agreement between APT and SPT performed with the same allergen or with other allergens (atopy status by definition). This is in apparent contradiction with the existing literature which found a strong prevalence of subjects positive to both types of tests in several papers concerning infants [23,24], children [6-8,14,25] and adults [3,15,26] with eczema or with food allergy [21,28]. Literature suggests that in subjects with food allergy positive results of each of the two tests (atopy patch tests or skin prick tests) are at least in part linked to the time elapsing between exposure and the appearance of clinical symptoms: SPT are typically larger [28] or more frequently positive [22-25] in patients in whom symptoms appear early after the exposure, whereas positive APTs are more frequently found in subjects with late symptoms [2,23,25]. In general, however, insofar as they both largely explore the pathogenesis of biological situations that share important elements, specifically cellular responses mediated by the immunoglobulin molecule IgE [9,13,16,18], we should expect a strong prevalence of subjects positive to both tests. The fact that we did not find this overlapping at the epidemiological level in unselected school children suggests that only in casuistic the severity of the symptoms or the intensity of positive test responses differ in our populations and in the clinical cases reported in the foregoing cited papers.

In conclusion, our study, the first robust epidemiological evaluation of the APT in two paediatric populations, clarifies some points but leaves several others unanswered, for example, the reproducibility of a positive result in the immediate and long term, the clinical meaning of weak or questionable results, and the prevalence of positive tests in children younger or older than the 10-year-olds we studied. Most important, it leaves for future studies the task of understanding further the biological and clinical meaning of the retarded skin reactivity expressed by atopy patch tests.

REFERENCES

1. Mitchell EB, Crow J, Chapman MD, et al. Basophils in allergen-induced patch test sites in atopic dermatitis. Lancet 1982; 1: 127-30.
2. Darsow U, Vieluf D, Ring J. Evaluating the relevance of aeroallergen sensitisation in atopic eczema with the atopy patch test: a randomized, double-blind multicenter study. J Am Acad Dermatol 1999; 40: 187-193.
3. Darsow U, Laifaoui J, Kerschenlohr K, et al. The prevalence of positive reactions in the atopy patch test with aeroallergens and food allergens in subjects with atopic eczema: a European multicenter study. Allergy 2004; 59: 1318-1325.
4. Niggemann B. The role of the atopy patch test (APT) in diagnosis of food allergy in infants and children with atopic dermatitis. Pediatr Allergy Immunol 2001; 12 (Suppl 14):37-40.
5. Turjanmaa K, Darsow U, Niggemann B, et al. EAACI/GA2LEN position paper: Present status of the atopy patch test. Allergy 2006; 61: 1377-1384.
6. Roehr CC, Reibel S, Ziegert M, et al. Atopy patch tests, together with determination of specific IgE levels, reduce the need for oral food challenges in children with atopic dermatitis. J Allergy Clin Immunol 2001; 107: 548-553.
7. Strömberg L. Diagnostic accuracy of the atopy patch test and the skin-prick test for the diagnosis of food allergy in young children with atopic eczema/dermatitis syndrome. Acta Paediatr 2002; 91: 1044-1049.
8. Rokaitè R, Labanauskas L, Vaidelienè L. Role of the skin patch test in diagnosing food allergy in children with atopic dermatitis. Medicina (Kaunas) 2004; 40: 1081-1086.
9. Langeveld-Wildschut EG, Van Marion AM, Thepen T, et al. Evaluation of variables influencing the outcome of the atopy patch test. J Allergy Clin Immunol 1995; 96: 66-73.
10. Heine RG, Verstege A, Mehl A, et al. Proposal for a standardized interpretation of atopy patch test in children with atopic dermatitis and suspected food allergy. Pediatr Allergy Immunol 2006; 17: 213-217.
11. Mehl A, Rolinck-Werninghaus C, Staden U, et al. The atopy patch test in the diagnostic workup of suspected food-related symptoms in children. J Allergy Clin Immunol 2006; 118: 923-929.
12. Darsow U, Vieluf D, Ring J. Atopy patch test with different vehicles and allergen concentrations: an approach to standardisation. J Allergy Clin Immunol 1995; 95: 677-684.
13. Bruijnzeel PL, Kuijper PHM, Kapp A, et al. The involvement of eosinophils in the patch test reaction to aeroallergens in atopic dermatitis: its relevance for the pathogenesis of atopic dermatitis. Clin Exp Allergy 1993; 23: 97-109.
14. Niggemann B, Reibel S, Wahn U. The atopy patch test (APT) – a useful tool for the diagnosis of food allergy in children with atopic dermatitis. Allergy 2000; 55: 281-285.
15. Ingordo V, Andria GD, Andria CD, et al. Results of atopy patch tests with house dust mites in adults with “intrinsic” and “extrinsic” atopic dermatitis. JEADV 2002; 16: 450-454.
16. Holm L, Matuseviciene G, Scheynius A, et al. Atopy patch test with house dust mite allergen an IgE-mediated reaction? Allergy 2004; 59: 874-882.
17. Langeveld-Wildschut EG, Bruijnzeel PL, Mudde GC, et al. Clinical and immunologic variables in skin of patients with atopic eczema and either positive or negative atopy patch test reactions. J Allergy Clin Immunol 2000; 105: 1008-1016.
18. Johansson C, Eshaghi H, Tengvall Linder MT, et al. Positive atopy patch test reaction to Malassezia furfur in atopic dermatitis correlates with a T helper 2-like peripheral blood mononuclear cells response. J Invest Dermatol 2002; 118: 1044-1051.
19. Kerschenlohr K, Decard S, Przybilla B, et al. Atopy patch test reactions show a rapid influx of inflammatory dendritic epidermal cells in patients with extrinsic atopic dermatitis and patients with intrinsic atopic dermatitis. J Allergy Clin Immunol 2003; 111: 869-874.
20. Spergel JM, Beausoleil JL, Mascarenhas M, et al. The use of skin prick tests and patch tests to identify causative foods in eosinophilic esophagitis. J Allergy Clin Immunol 2002; 109: 363-368.
21. De Boissieu D, Waguet JC, Dupont C. The atopy patch test for detection of cow’s milk allergy with digestive symptoms. J Pediatr 2003; 142: 203-205.
22. Majamaa H, Moisio P, Holm K, et al. Cow’s milk allergy: diagnostic accuracy of skin prick and patch tests and specific IgE. Allergy 1999; 54: 346-351.
23. Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. J Allergy Clin Immunol 1996; 97: 9-15.
24. Kekki OM, Turjanmaa K, Isolauri E. Differences in skin-prick and patch-test reactivity are related to the heterogenity of atopic eczema in infants. Allergy 1997; 52: 755-759.
25. Breuer K, Heratizadeh A, Wulf A, et al. Late eczematous reactions to food in children with atopic dermatitis. Clin Exp Allergy 2004; 34: 817-824.
26. Seidenari S, Manzini BM, Danese P, et al. Positive patch test to whole mite culture and purified mite extracts in patients with atopic dermatitis, asthma and rhinitis. Ann Allergy 1992; 69: 201-206.
27. Guler N, Kirerleri E, Tamay Z, et al. Atopy patch testing in children with asthma and rhinitis symptoms allergic to house dust mite. Pediatr Allergy Immunol 2006; 17: 346-350.
28. Vanto T, Juntunen-Backman K, Kalimo K, et al. The patch test, skin prick tests, and serum milk-specific IgE as diagnostic tools in cow’s milk allergy in infants. Allergy 1999; 54: 837-842.

BLOOD BORNE INFECTIONS IN STOMATOLOGICAL PRACTICE: SYNTHESIS OF ANSWERS ACCORDING TO THE FIELDS OF INTEREST BASED ON THE QUESTIONNAIRE FOCUSING ON ESTIMATION OF BLOOD BORNE INFECTIONS RISK IN STOMATOLOGICAL PRACTICE

[1]Elena Nováková, [2]Ivan Snopek, [1, 5]Pavel Hubočan, [2]Dagmar Mullerová, [1]Jana Kompaníková , [3]Nils Skaug, [4]Eugenia Aura Negut

[1]Ústav mikrobiológie a imunológie, JLF UK, Martin, Slovakia, [2] Labmedto, s. s r.o., Neštátne zdravotnícke zariadenie laboratórnej medicíny, Topoľčany, Slovakia, [3]Dept. of Microbiology, University of Bergen, Stomatological Faculty, Bergen, Norway, [4]Cantacuzino Institute, HIV Reference laboratory, Bucarest, Romania, [5]Regionálny úrad verejného zdravotníctva, Čadca, Slovakia

Abstract

Contact with blood, exudate or transudate is one of the major mechanism involved in the spread of infectious diseases. Stomatological workplaces have always been considered as very riskful for acquiring the professional and also hospital infection by blood borne pathogens. The questionnaire focusing on estimation of blood borne infections risk in stomatological practice was used as a method for collecting data that made a base for a retrospective-prospective analysis and their processing. The existing legislative, health care and public health rules and the knowledge group of respondents are adequate for prevention of blood borne infections in stomatological practice if followed thoroughly. The synthesis of results received from questionnaire analysis shows the impact and use of these knowledge in the stomatological practice.

INTRODUCTION

Contact with blood, exudate or transudate is one of the major mechanism involved in the of infectious diseases. Bleeding injuries interrupting skin or mucous membranae, hyperaemia of cutaneous and mucous surfaces during inflammation or hypersensitivity, minimal injuries as consequences of thorough and repeated washing and disinfecting solution application can cause decrease of barriere mechanism in non specific immunity line and can be the portal of entry of blood borne pathogens. Sharp injuries, absence of preventive measures or their application, mistakes in treating and nursing techniques are other risk factors. Accidental injuries are recorded by the majority of health care workers during their professional career, and they represent possible exposition to hepatitis B virus (HBV), hepatitis C virus (HCV), HIV and some others (1,7,8). Any infectious pathogen that is present during the pathogenesis of the disease in the blood (the period of viraemia, bacteraemia, fungaemia, parasitaemia) is a possible risk of blood borne infection in case of the contact of nonimmune person with the blood or blood derived / blood contaminated body fluids. Stomatological workplaces have always been considered for highly risky for acquiring the professional and also hospital infection with blood borne pathogens (3). Stomatological clinic is a relatively independent functional unit with a relatively closed number and steady characteristics of patients that seek medical treatment repeatedly. It is regarded as dangerous for its exposition to blood and body fluids, with frequent surgeries or invasive procedures and is relatively indepedant in sterilization processes and sterile material supply. Risk of transmission of blood borne infection from patient to health care workers, or vice versa, or cross infection of patients is thus very high.

Estimation of the real burden of risks of blood borne infections and the crucial moments in their transmission were the focal subjects of the international project of European Union International Cooperation Programme Copernicus (INCO Copernicus) and are also the main aims of this presentation. The group of Slovak workers was identified according to the methodology of the mentioned project so that it fits to its requirements for demographic characteristics and was found comparable with other involved countries.

Address for correspondence:
MUDr. Elena Nováková, PhD., Department of Microbiology and Immunology, Commenius University, Jessenius Faculty of Medicine, Sklabinská 2, 037 01 Martin, Slovak Republic
Tel.: ++ 421 43 4239038

MATERIAL AND METHODS

All stomatologists of the district of Čadca, Topoľčany, Žilina and Spišská Nová Ves were addressed in written way. The envelope with accompanying letters of introduction and information, questionnaire, injuries registration form and steriliser monitoring recording form was sent to each of them. Cohort was represented by individually working stomatologists in outpatient departments or chief stomatologist in the hospital department. The questonnaire was prepared to involve all necessary epidemiological and demographic information. Full text in Slovak and English is available in corresponding author. The information from questionnaire were registred in PC Excell programme and programme EPI INFO 5. The questionnaire was used as a method for collecting essential data. Retrospective-prospective analysis was used for processing of these data. Results were statistically processed and analysed using EPI INFO 5. The frequency and ratio of answers were calculated and a synthesis of answers according to the fields of interest was done and is presented in a form of tables prepared by a Roumanian counterpart and adopted by all project partners so that the results can be easily compared between cooperating countries (E.A.Negut). These methods were used to meet the requirements and the main aim of this scientifical project that was to gather available information from stomatological working places with possible risk of blood borne infections transmission, analyse them according to the fields of interest, describe the situation, identify possible riskful points in stomatological practice and suggest further measures to improve the situation in this sensitive field.

RESULTS

Results are given in the tables 1-5:

Tab.1. Demographic data

Question	% of respondents	Text
Q3	35	Males
Q4	66	Married
Q2	57	44-53 year old
Q5	78	Over 20 year of experience in practice
Q6	87	General stomatologist – without specialty
Q9	51	Treat 10 – 19 patients daily
Q9	42	Treat 20 – 29 patients daily
Q7	33	Working in the rural area
Q8.1	62	The only stomatologist working in the place
Q8.3	93	Have no dental hygienist in the office
Q8.3	100	Have at least 1 dental nurse in the office

Tab. 2 Data regarding the knowledge related to blood borne transmissible infections

Question	% of respondents	Text
Q10	43	Had 0 hour of education in the past 3 years
	33	Had 1-2 hours of education in the past 3 years
Knowledge about hepatitis B		
Question	% of respondents	Text
Q 27	62	Know that majority of all persons with HBV were unaware of exposure
Q 25	62	Know that after accidental percutaneous or mucus blood exposure person should be tested for anti HBs without regards to vaccination status
Q 26	52	Give exact indication of revaccination

Q 20	23	Indicate correct vaccination schedule
Knowledge about hepatitis D		
Question	% of respondents	Text
Q 31	79	Could not give any answer about mortality of HDV
Q 30	9	Know that vaccination against HBV protects also against HDV
Knowledge about hepatitis C		
Question	% of respondents	Text
Q33	37	Hepatitis C symptoms mixed with those of hepatitis B
Q 34	60	Give correct estimation of prognosis
Q 39	42	Give a correct estimation of mortality rate
Q 38	40	Give a correct estimation of virus infectivity
Q 36	63	Know all routes of transmission
Q 35	90	Indicated correctly "risk group" for hepatitis C
Q 37	71	Think that HCV may be transmitted during the dental treatment
Knowledge about HIV		
Question	% of respondents	Text
Q 62	20	Correctly estimate that medical histories and physical examinations cannot identify all carriers of hepatitis and HIV
Q 63	65	Correctly estimate that most HIV infected persons are without symptoms
		for a long period (about 10 years)
Q 64	40	Correctly think that pure saliva cannot readily transmit HIV
Q 60	49	Correctly know that HIV infection is less infectious then hepaititis B
Q 122	1,4	Correctly estimates the risk from needle stick injury to less than 1%
Q 61	48	Correctly assert that infection control practices for hepatitis B are
		adequate for protection against HIV
Q 59	44	Assert that they treat all patients as if they have hepatitis B or HIV
Q 55	71	Think that additional infection control procedures necessary to treat
		HIV patient will be a financial burden for their practice
Q 57	81	Think that the type of personal barrier protection they use depends
		upon the patients infectious state
Q 58	35	Think that they can safely treat a person with HIV infection in their
		office
Stomatologist involvement to co workers protection		
Question	% of respondents	Text
Q 21	74	Refer that nurses working with them are vaccinated
Q 22	91	Informed the coworkers of the importance of vaccination
Q 23	84	Do not know why the nurses working with them have not been
		vaccinated against hepatitis B
Q 24	46	Agree to pay the cost of vaccination for the nurse

Tab. 3. Extent to which knowledge of infections is reflected in professional practice.

Attitude towards hepatitis B vaccination		
Question	% of respondents	Text
Q 12	78	Were vaccinated against HBV
Q 14	73	Declare correct vaccination schedule with 3 doses
Q 15	74	Declare the correct application method
Q 17	8	Indicate that the effict of their vaccination was serologically proved

Q 18	50	Declare their revaccination (majority for long time interval from last dose)
Q 19	8	Boostered because of exposure to high risk patient
Attitude towards the potential risk represented by patients from the risk group		
Question	% of respondents	Text
Q 124	21	Have treated patients with hepatitis B
Q 125	4	Have treated patients with hepatitis C
Q 126	3	Have treated patients with HIV infection
Q 127	7	Have treated patients of risk groups
The importance of being aware of the patient's "risk status"		
Question	% of respondents	Text
Q 43	76	Feel the importance of information about the patient's "risk status"
Q 45	97	Feel the above mentioned importance in case of HIV infection
Q 80	75	Assert the medical history taken from their patients include question(s) about blood borne infections
Q 124-127	60-70	Do not know if some of their patients belongs to the risk group
Readiness to treat patients from the "risk group":		
Question	% of respondents	Text
Q 71	45	Agree fully or to a certain degree to treat HIV infected patient
	27	Strongly disagree to treat HIV patient
Q 65	66	Agree fully or to a certain degree to treat drug addicts
Q 67	72	Agree fully or to certain degree to treat persons who received non tested blood
Q 68	58	Agree to treat patient with sexually transmissible diseases (STDs)
Q 69	63	Agree to treat patients with hepatitis B
	8	Strongly disagree to treat patients with hepatitis B
Q 70	65	Agree to treat patients with hepatitis C
	10	Strongly disagree to treat patients with hepatitis C
Q 29	41	Feel comfortable about treating HBV infected patients in their office
	45	Feel apprehensive about treating HBV infected patients in their office
Acceptability of risk group patients		
Question	% of respondents	Text
Q 50	93	Think that they will be included in the risk group if they treat HIV infected patient
Q 72	55	Would accept to be treated by colleague who is treating HCV and HBV infected patients
Q 73	44	Would accept to be treated by colleague who is treating HIV/AIDS patients
Q 46	55	Think that patient is entitled to know about dentist's epidemiologically important health conditions
Q 51	86	Consider that the collaboration with the team would become difficult if they would treat HIV positive patient
Q 52	73	Consider that the other patients will refuse him as stomatologist if he treats HIV patients
Q 44	96	Dentists will ask patient to be serologically tested in case of susceptibility of patient's risk status

Tab. 4. The control of cross-infections in dental practice. Data reflecting extent to which the general infection control rules are observed.

Knowledge of the rules		
Question	% of respondents	Text
Q 78	80	Know the universal precautions
Q 75	89	Know how to follow the official rules for infectious disease control
Q 76	71	Have guidelines referring infection control in office
Q 77	81	Declare that the guidelines are followed in their office
The dentists declare that they use or would use" special" measures:		
Question	% of respondents	Text
Q 81	40	In case of HIV infected patient treatment
Q 82	53	In case of HBV infected patient treatment
Q 55	73	Declare that special measures would be costly
Data regarding self-protection and the protection of auxiliary personnel		
Question	% of respondents	Text
Q 113	77	Wear gloves
Q 110	92	Wear mask
Q 99	73	Wear protective glasses
Q 95	16	Declare latex allergy (even if not tested)
Q 105	10	Use antiseptic solution for mouth rinsing
Q 115	81	Use saliva aspirator
Q 114	37	Use high capacity aspirator
Q 116	21	Use rubber protective cloth
Q 117	86	Disinfect dental impressions and prostheses before being sent to technician to laboratory
Q 119	72	Disinfect prostheses after removal from mouth
Q 106	66	Have containers for disposable sharps
Q 108	59	Recap manually anesthesia needles
Q 109	25	Recap with the aid of an appliance or clip needles
Data regarding the patient's protection		
Question	% of respondents	Text
Q 98	100	Wash their hands before each patient
Q 100	90	Wash their hands after using the gloves
Q 101	48	Change the gloves after each patient
Q 111	7	Change mask after each patient
Q 102	55	Let the water in the unit run after each patient
Q 118	46	Disinfect dental impressions and prostheses after receiving them from the laboratory
Q 120	38	Disinfect dental impressions and prostheses before placing them in the mouth
Q 104	38	Use disposable instrument
Q 103	13	Sterilize the contra angles and turbines after each patient
Q 93	10	Accept conditionally boiling water for sterilization
Q 91	100	Clean instruments mechanically prior sterilization

Tab. 5. Sterilization monitoring

Question	% of respondents	Text
Q 83	94	Use dry heat sterilizers
Q 86	23	Use steam autoclaves
Q 90	88	Use also other types of sterilization (UV, chemical)
Q 84	14	Monitor heat sterilizers with chemical indicators
Q 85	75	Monitor heat sterilizers with biological indicators
Q 87	16	Monitor autoclaves with chemical indicators
Q 88	44	Monitor autoclaves with biological indicators

DISCUSSION

Use of questionnaire as working method is always to be taken as subjective. The control group is not available in this method of work and there is always the risk, that the group of responders differs in characteristics and proportion of obtained answers from the group of non respondents.

Demographic data reflect well that the population of stomatologists is formed mostly of women and is getting older. More than 80 % are working more than 20 years, but more that 30 % are over 60 years. As the majority of stomatological working places are privat outpatient departments and the professional relations changed so that the stomatologist is the one who executes the professional work, who is the manager, economist, organizer, employer with all professional, economic and manager's responsibilities that bring the lack of time for continuous education and stress that force to neglect fields that seem to be not extremely urgent.

The risk of professional and hospital infections with blood borne pathogens is relatively low because of the low prevalence of HIV and HCV infection in Slovak population. HBV infection is more common but the transmission is controlled by specific preventive measures (vaccination) (4,5). The risk of contracting HIV infection from HIV contaminated needle stick injuries is generally oversized by respondents and general population. On the other hand it is almost not taken in account as possible. Anyway, if possible, it is taken very seriously. This statement is supported by the requirement that in case of HIV infected patients specialized clinics could be required if the patient is without signs of the disease or in case of AIDS patients. The prevalence of HIV infection is still not being a public health problem. It is until now very low in general population and in stomatologists: In our study it was found 0 in stomatologists. The subjectively reported occurrence of HIV infected patients could not be confirmed or laboratory objectivised in our study. Risk of blood borne infections is anyway not negligable because of lack of strict following of universal precautions and of good hospital practice rules for infectious diseases (2).

Data regarding the knowledge related to blood borne transmissible infections show that the absence of continuous and update education can increase the risk because new information possibly of increasing prevalence, new infections or new characteristics should be regularly forwarded to those who are mostly concerned. Certain knowledge and attitudes can themselves be dangerous. Between them the reasons for "non-vaccinated" such as "lack of time", belief that they "know patients well enough to be protected", "the vaccine is not available", "I haven't supplies", "it is riskful" are those that can be taken as a threat. On the other hand the statement that "the equipment gives sufficient protection" can be taken as a theoretical goal in prevention of blood borne infections, as the exposure needs not necessarily to be overt and usually is not and the stomatologist is not always aware of it. Extent to which knowledge of infections is reflected in the professional practice cannot be evaluated only from the questionnaire and has to be objectivised. Generally the routine (not routineous) good hospital practice approaches with reasonable algorithm followed strictly by the team can be a good way how to assure the anti infectious protection. Anyway injuries and accidents have always been happening. The analysis of them and appropriate measures implementation can be the base how to ameliorate the team in

process. The first step for analysis is the correct reporting and notification. But after the accident, majority of stomatologists do not notify or record it.

The control of cross-infections in the dental practice and sterilization monitoring are the fields that can be objectivised by regular sterilisation process and sterility control and by monitoring and identification of hospital and professional diseases (6,11). The serological testing results show that 32 of 48 stomatologists that could be sampled (67.7 %) look like being vaccinated (HBcAb- and HBsAb+). For others who declare vaccination and have no detectable antibody levels the explanations could be:"non responders" or "the vaccination performed more than 10 years ago (5% in study group of stomatologists according to questionnaire). Hepatitis B vaccination coverage is to grow as students of stomatology are obligatory vaccinated. This should not be taken as a measure that solves the problem of blood borne infection in stomatological practice – as hepatitis B is not the only (even if in our population the most prevalent) blood borne infection.

The automated sterilization process seems to be one of the solid support in blood borne infection control as all sterilizers tested in external control were showed to be working efficiently (9,10). Some historical knowledge are still being used (10 % declare boiling water as the method of sterilization) and newer information are not being accepted. 7 % of respondents do no realize that after 20 minutes the mouth mask is so humid that it can transmit infection. This corresponds (as one example) with the lack of continuous education.

CONCLUSIONS

At this level of results proceeding, the authors are aware of the limitations of presented results, as they were gathered from questionnaires, that is rather a method relying on individual answers and thus with a burden of subjectivity and false answers. The presented synthesis is the base for thorough and multivariable analysis followed by comparison with results of other cooperating project partners. The results show the need of well-organized postgraduate education in selected topics. Changes in organization of work could save time for better surveillance of injuries. Information flow in reporting of infectious diseases (based on computerization of departments) should be oriented also to stomatologists. Anyway the existing legislative, health care and public health rules and majority of knowledge are adequate for prevention of blood borne infections in stomatological practice if followed thoroughly.

REFERENCES

1. Cottone JA, Puttaiah R. Hepatitis B virus infection. Current status in dentistry. In: Glick M. Infectious diseases and dentistry. Dent Clin North Am. 1996; 40 : 293 - 308.
2. Downs AM, Heisterkamp SH, Brunet JB.: Reconstruction and prediction of HIV/AIDS epidemic among adults in the European Union and in the low prevalence countries of central and Eastern Europe AIDS 1997; 11: 649-62.
3. Guidelines for Infection Control in Dental Health Care Setting 2003. MMWR 2003 ; 52 (RR 17) :1-61.
4. Hagberg M, Hofmann E. Occupational Health for Health Care Workers. Landsberg: Ecomed, 1995.
5. Hudečková H, Szilágyiová M. Vírusová hepatitída B – profesionálne ochorenie u zdravotníckych pracovníkov v oblasti Turca v rokoch 1976-2000. České Pracov Lék 2002 ; 3: 134-136.
6. Kneiflová J. Dezinfekční přípravky II. Praha: Kneifl, 2001.
7. Lavanchy D. The threat to public health hepatitis C. Res Virol 1997 ; 148:143-45.
8. Montebugnoli L, Dolci G. Anti-HCV antibodies are detectable in the gingival cervicular fluid of HCV positive subjects. Minerva Stomatol 2000 ; 49: 1-8.
9. Pazdiora E. Lékařské nástroje a zdravotnícke pomůcky. In: Šrámová H. Nozokomiální nákazy II. Praha: Maxdorf, 2002 ; p. 303.
10. Sepkowitz KA. Occupationally acquired infections in health care workers. Part II. Ann Intern Med 1996 ; 125. 917-28.
11. Volná F. Dezinfekcia a sterilizácia – teória a prax. Žilina: Vrana 1999.

ERRATA

The paper: „Kaposi's sarcoma versus Kaposi-like hemangiomatous lesions" (Acta Medica Martiniana 7/1, 17 - 22 pp. was published with an error: Legends to Figure 1 and 2 were mutually replaced. The editors apologize to the authors for this error.