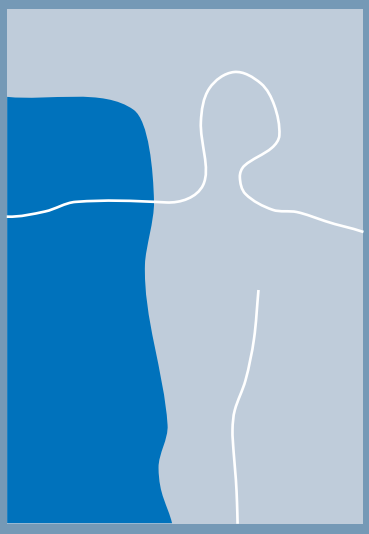

# Acta CHIRURGICA Latviensis

2012 (12)

# ACTA CHIRURGICA LATVIENSIS, 2012 (12)

ISSN 1407-981X

Electronic version – www.versita.com





RĪGAS STRADIŅA UNIVERSITĀTE

**Layout** Lita Zvaigznite

**Publishers** Riga Stradins University
Latvian Surgeon`s Association
Latvian Pediatric Surgeon`s Association

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Typeset and printed by „ULMA" Ltd

The number of copies – 1000

Project "Promotion of an international cooperation activities of the Riga Stradins University in the science and technologies"
(agreement No. 2010/0200/2DP/2.1.1.2.0/10/APIA/VIAA/006)

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10.2478/v10163-012-0001-0



*ORIGINAL ARTICLE*

# Key Factors Contributing to Quality of Life for Breast Cancer Patients in Latvia: Supplementing Quantitative Surveys with Qualitative Interviews

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**Summary**
**Introduction.** Health related quality of life is a much debated topic in medicine with much quantitative and qualitative research contributing to the understanding of how to improve the lives of patients, yet little has been published in relation to the quality of life of Latvian breast cancer patients.
**Aim of the Study.** To gather base measurements of subjective and objective quality of life factors for breast cancer patients in Latvia and discover which key factors contribute most to quality of life of Latvian breast cancer patients at the start of treatment.
**Materials and Methods.** This paper presents data collected from April 2010 to June 2011 at the Pauls Stradins Clinical University hospital on key factors influencing quality of life for breast cancer patients: health and physical well-being; state of surroundings and environment; social support and functionality; financial state, employment and leisure. Quantitative survey material has been supplemented with insight from qualitative in-depth interviews to better explain the objective and subjective implications for breast cancer patients' quality of life.
**Results.** Interviewed breast cancer patients rated their quality of life as being average or good at the beginning of treatment. Negative factors contributing to lowered quality of life were mainly linked to patient financial, social and emotional state at the first weeks of treatment and correspond to previous research done in Latvia on quality of life issues.
**Conclusions.** Further follow-up surveys will contribute to the evaluation of breast cancer patients' needs while undergoing treatment to further improve treatment strategies, especially if validated quality of life measurement surveys were to be implemented in Latvian hospitals.
**Key words:** quality of life, breast cancer, quantitative survey, qualitative interviews

## INTRODUCTION

Current medicine recognizes that it is crucial to understand not just physiological imperatives, but also uncover social and psychological needs of patients to treat illness. To understand the development of particular ailments and overall improve population health - much attention is being paid to aspects contributing to patient quality of life as well as their subjective perception and attitudes towards treatment (1, 9).

Cancer affects one in three of Europe's population including Latvia's inhabitants. Incidence of cancers is dependent on many factors, including social, economic, geographic, and demographic. Successful cancer control programs include promotion of healthy lifestyles, preventive screening, high-quality treatment, palliative care, as well as psychological and social aspects. Yet in prior years, research has shown that the Latvian health care system is very taxing on the population with people having to put off medical examinations due to financial hardship which leads to a high rate of late detection for oncological diseases (3, 8, 13). Low income and limited access to health care contributes to reduced overall quality of life, which is characterized by the individuals' access to a certain level of consumption, range and quality of social services, ability to live a long and healthy life. Based on previous research Latvians can be characterized as being partially satisfied with their lives and their quality of life is impacted the most by the state of material prosperity, security, family and vocation (8). In medicine quality of life measurements help to assess which treatment methods improve and which reduce a patient's quality of life; when to choose palliative and when clinical treatments; evaluate the dynamics of different treatment methods to determine direct effects on patients' quality of life during treatment and after recovery (6, 12). Such measurements are useful to develop new treatment strategies, evaluate the efficiency of existing treatments, as well as determine which treatments are best suited for a patient or group of patients (15, 28). Generic surveys on population quality of life (8, 13) and more detailed qualitative and quantitative research on general population quality of life (2, 20) and health related quality of life for particular maladies (10, 18, 26) have been previously conducted in Latvia. Yet as of today, no standardized measures are being used in Latvia for breast cancer patient quality of life evaluation.

This paper presents data on patient quality of life from the on-going research, including analyses of data obtained from quantitative surveys and qualitative

in-depth interviews. Health related quality of life is analysed in light of the patient subjective evaluations of key standardized factors (5, 24) contributing to quality of life while taking into consideration the bio-psychological model of health (4). This research project is unique and no prior research on breast cancer patient social factors of this scale has been undertaken in Latvia.

## AIM OF THE STUDY

Aim of the study is to gather base measurements of subjective and objective quality of life factors for breast cancer patients in Latvia and to discover which key factors contribute most to quality of life of Latvian breast cancer patients at the start of treatment.

## MATERIALS AND METHODS

Health related quality of life is a term used to explain the complex state of patients by investigating physical, psychological and social aspects that influence them. Quality of life is determined by a person's experience, beliefs, expectations and perceptions (14, 27). Quality of life must be analysed through both objective factors and subjective interpretations, thus quantitative methods are used to establish base measures, and qualitative methods to explain underlying processes (7).

This paper is based on a survey of breast cancer patients conducted from April 2010 until June 2011, during this time 150 interviews were collected and among those 135 answers to the quality of life section. Also during this period 9 hereditary breast cancer gene carriers were interviewed from May 2011 until September 2012. This study was approved by the Ethical Comittee of Riga Stradins University and all patients involved signed informed consent forms.

The quantitative interviewer administered survey of breast cancer patients was conducted at the Pauls Stradins Clinical University Hospital, where all patients after a mastectomy surgical operation were approached, informed about the study and asked to participate. In most cases the survey was realized on the next day after the operation. The 10 page questionnaire was constructed to include crucial information on patient demographics, reproductive health, life-styles, experience with and information on medical check-ups, as well as quality of life. A new generic quality of life tool was modeled after the WHOQoL-BREF measurement system (22, 29). This part of the questionnaire consists of questions evaluating overall quality of life, state of health, emotional state and 4 groups of standardized factors – a) health and physical state, b) functional and social state, c) financial state and leisure time, d) surroundings/environmental state. Additional questions are included to evaluate the importance of the factors used for determining the quality of life of each patient.

The qualitative in-depth interviews were carried out with hereditary breast cancer gene carriers from different typological groups and cities in Latvia after random selection from the BRCA gene carrier database. The interviews took place at a hospital or in patients' homes. An audio recording was made of each interview and a semi structured questionnaire was used as the research instrument.

Descriptive statistical data in this paper are presented as means, frequencies, proportions and percent of included patients was calculated by MS Office Excel and SPSS. Reported confidence intervals and standard deviations are shown with '±' and were calculated at a 95% confidence level, correlations are reported for Pearson's $r$ value and statistical significance is reported with the two tailed $p$ value.

## RESULTS

### *Respondents' profile*

The national cancer register in the year 2010 recorded 1,057 breast cancer patients (25) and at Pauls Stradins Clinical University hospital in 2010 a total of 195 breast operations were performed. During the period between April 2010 and June 2011 a total of 150 patients were interviewed, one was male and the rest female. The age of patients ranged from 28 to 86 years with mean age of 62 (±13; standard error 1). Approximately half of the patients were the age of 65 and older (48±8%), and the other half younger. Majority of patients (47.3±8%) had a general secondary or vocational secondary education, more than one third (34.7±7.6%) had a university degree, while 18±6.1% had primary and lower education, yet among patients 65 and older this was characteristic of 33±7.5% (a moderate correlation between age and level of education; $r$=0.441, $p$=0.00000002). Nearly half of patients (47±8%) were retired and living from state pensions, while 34±7.6% were working, 5±3.5% were housewives and 8±4.3% were permanently disabled. Compared to the national level of 17% on average, only 4% of respondents were unemployed.

On average the minimum consumer basket per capita as recorded by the Central Statistical Bureau of Latvia during the period of data acquisition was 169 LVL (16). The average income per family member each month noted by the patients undergoing breast cancer surgery was 187.50 LVL (±107.64 LVL, standard error 9.20), though more than half (60%) of the patients in this study aged 65 and older were living below the state minimum consumer basket (small correlation between age and income level; $r$=-0.169, $p$=0.047).

### *Quality of life*

Quality of life for patients was measured using an original questionnaire as part of a larger survey. From 150 surveyed patients, 135 replied to the quality of life questionnaire section. Additional insight was gained during in-depth interviews with hereditary breast cancer patients.

Overall the interviewed breast cancer patients rate their quality of life as being average or good at the beginning of treatment (see Table 1). A 3$^{rd}$ of the interviewed patients thought that their health prior to the treatment was good, while from the three overall ratings the emotional state received the most critical evaluation.

Negative factors contributing to lowered quality of life ratings were mainly linked to breast cancer patient financial and emotional state at the first weeks of treatment. Greater insight was gained through in-depth interviews.

**Table 1. Quality of life assessment in the last 2 weeks prior to surgery - Overall, Health and Emotional state**

| | Very bad | Bad | Partial | Good | Very good | NA |
|---|---|---|---|---|---|---|
| How would you rate your quality of life? | 5.9%±4,0% | 14.1%±5,9% | 33.3%±8,0% | 38.5%±8,2% | 5.2%±3,7% | 3.0%±2,9% |
| How would you rate your state of health? | 8.9%±4,8% | 23.0%±7,1% | 28.1%±7,6% | 34.8%±8,0% | 3.0%±2,9% | 2.2%±2,5% |
| How would you rate your emotional state? | 16.3%±6,2% | 21.5%±6,9% | 26.7%±7,5% | 28.1%±7,6% | 4.4%±3,5% | 3.0%±2,9% |

(N=135, confidence level 95%)

The following part of this article focuses on the analysis of factors most influencing quality of life for patients after diagnosis of breast cancer where quantitative survey data has been supplemented with qualitative interview material.

***Health and physical well-being***

Previous research established that Latvians do not associate care for quality of life with care for health - during interviews health care specialists and citizens admitted that health is a potential aspect that can be sacrificed through hard work to attain a qualitative life (19). Recent research shows that 4.6% of respondents characterised their health as *very good*, 44% as *good* and 16.2% in total as *bad* or *very bad*. When considering age groups it must be noted that among those aged 65 and over 45% rated their health as *bad* or *very bad* (13).

**Table 2. Quality of life assessment in the last 2 weeks prior to surgery - Health and Physical state**

| | Not at all | A little | Partially | A lot | Completely | NA |
|---|---|---|---|---|---|---|
| To what extent did physical pain prevent you from doing what you need to do every day? | 50.4%±8,4% | 17.0%±6,3% | 11.1%±5,3% | 8.9%±4,8% | 11.1%±5,3% | 1.5%±2,0% |
| How well were you able to get around physically? | 3.0%±2,9% | 12.6%±5,6% | 8.1%±4,6% | 11.1%±5,3% | 65.2%±8,0% | - |
| How satisfied were you with your sleep? | 14.8%±6,0% | 18.5%±6,6% | 11.9%±5,5% | 23.0%±7,1% | 31.1%±7,8% | 0.7%±1,4% |
| How much did you need the support of others to do what you need to do every day? | 68.9%±7,8% | 9.6%±5,0% | 8.1%±4,6% | 3.0%±2,9% | 5.2%±3,7% | 5.2%±3,7% |
| How well were you able to concentrate? | 1.5%±2,0% | 9.6%±5,0% | 13.3%±5,7% | 11.9%±5,5% | 60.7%±8,2% | 3.0%±2,9% |
| Did you have enough energy for everyday life? | 6.7%±4,2% | 12.6%±5,6% | 22.2%±7,0% | 13.3%±5,7% | 43.0%±8,4% | 2.2%±2,5% |
| How satisfied were you with your physical appearance? | 3.7%±3,2% | 7.4%±4,4% | 24.4%±7,2% | 23.0%±7,1% | 17.0%±6,3% | 24.4%±7,2% |

(N=135, confidence level 95%)

In our survey *health* was rated as the most important of factors influencing quality of life with 92.2% patients rating it as absolutely important, yet *access to health services* was absolutely important only to 54% of patients (see Table 2 and 6). When asked to rate their health in the last few weeks, 3% of patients said their health was *very good*, 1/3 rated it as *good* (34.8%) while 31.9% thought it was *bad* or *very bad*. Among patients 65 and older health was rated as *bad* or *very bad* by 37±12% (a small correlation; $r$=-0.243, $p$=0.004). Half of the patients (50.4%) noted not suffering from any pain at all, but the majority (72±7%) was suffering from other chronic health problems prior to the diagnosis of cancer. Nearly all patients that did not have prior chronic illnesses still suffered from some sleep loss (94.5%) and fatigue (97.3%) in the prior weeks to surgery due to an enhanced negative emotional state after receiving a diagnosis of cancer.

While health is of the utmost importance and 73±7% admitted to undergoing regular health check-ups, only 49±8% regularly made the annual gynecological examinations and 29±7% a mammography. Among these patients the majority (55±9%) admitted they had not gone for check-ups because there had been no symptoms and complaints. Despite the fact that people are aware that regular health check-ups can reduce the risk of cancer, most choose to visit the doctor only when they are confronted with the effects of the disease or if there are serious health problems. Patients related general confusion regarding available diagnostics and distrust of medical intervention.

*In a way I let it go – I really didn't want there to be anything, when I visited the oncologist after my gynaecologist sent me. He asked me – how do you feel? Well, how could I feel? Nothing was hurting. He said that there is something there, but then – don't worry, come back after half a year. […] His attitude was*

*so careless. But since I wanted to hear that there is nothing wrong, I left his office with a light heart. If I had known then that you can puncture and take a biopsy, I would have done it, if there had been any suggestion! How could I have known about this method?! (Woman, age 48)*

Complete satisfaction with their *outer appearance* was rated by only 17% with ¼ of patients not wanting or being able to give an answer, but in general *outer appearance* was not seen to be that important for quality of life with only 28% rating it as absolutely important (see Table 2 and 6). Among patients ≤44 *outer appearance* was rated the highest with 33% being completely satisfied, while for patients ≥65 more than a 1/3 chose not to answer this question. More than half (54%) of patients 44 and younger rate *outer appearance* as a completely important factor for quality of life after a mastectomy operation while for each older group the importance seems to decline, but due to the low number of responses for this particular question data can not be assumed statistically significant. Patients have a choice to undergo reconstructive surgery, but the expenses are often too great, and the physical distortion can put emotional strain on younger women.

*He accepts me as I am, but in quiet he has mentioned that I could have the reconstruction done. Of course, what man doesn't want a woman lying next to him with both breasts? We have talked about this, we have discussed that it is a difficult operation and that right now it costs a lot of money. (Woman, 44)*

***Social support and functionality***

**Table 3. Quality of life assessment in the last 2 weeks prior to surgery - Functional and Social state**

| | Not at all | A little | Partially | A lot | Completely | NA |
|---|---|---|---|---|---|---|
| How satisfied were you with your ability to perform your daily living activities? | 7.4%±4,4% | 5.2%±3,7% | 9.6%±5,0% | 14.8%±6,0% | 58.5%±8,3% | 4.4%±3,5% |
| How satisfied were you with your capacity to work to make a living? | 3.7%±3,2% | 2.2%±2,5% | 7.4%±4,4% | 10.4%±5,1% | 27.4%±7,5% | 48.9%±8,4% |
| To what extent did you feel your life to be meaningful? | 4.4%±3,5% | 3.7%±3,2% | 9.6%±5,0% | 18.5%±6,6% | 54.8%±8,4% | 8.9%±4,8% |
| How satisfied were you with yourself? | 5.2%±3,7% | 0.7%±1,4% | 16.3%±6,2% | 21.5%±6,9% | 45.2%±8,4% | 11.1%±5,3% |
| How satisfied were you with your personal relationships? | 0.7%±1,4% | 3.7%±3,2% | 6.7%±4,2% | 28.1%±7,6% | 54.1%±8,4% | 6.7%±4,2% |
| How satisfied were you with the support you got from your family and friends? | 3.0%±2,9% | 4.4%±3,5% | 8.9%±4,8% | 14.8%±6,0% | 66.7%±8,0% | 2.2%±2,5% |

(N=135, confidence level 95%)

The need for emotional and psychological support was acknowledged by 67±8% of patients. The need for emotional support was more prominent among older patients, respectively 59±11% among younger than 65 years and 75±10% among older patients (a small correlation; $r$=-0.136, $p$=0.095).

Research on breast cancer narratives shows that for most women the diagnosis of cancer is unanticipated (21). In such cases there is a great need for psychological support. The stress leading up to treatment is also reflected in our survey, 16% of patients evaluated their emotional state two weeks before surgery as *very low* (see Table 3).

*But the most important - contact with other people, because if you are alone, you get even more depressed. We need a lot of contact. (Woman, 53)*

*Personal relationships* were the second most important factor influencing quality of life of the patients with 81% seeing it as an absolute priority (see Table 6). Completely satisfied with support from family and friends were 66%.

*It is important to keep hold of yourself, think positive thoughts. Day to day it's important to see the people around you who understand and support you, help to reduce stress. (Woman, 52)*

*Ability to do housework* and *ability to be physically active* were evaluated with top most importance by more than half of the patients (respectively 68% and 59%) (see Table 6). Positive ratings were common for *functional* and *social state* (see Table 3). Patients noted that it is important to keep active.

*I knit and crochet different pretty crafts, I really like it when I can see the result of my work and teach other people. I go to the forest, and keep a garden, so you might say that I have bad thoughts very rarely since I'm always busy. (Woman, 53)*

***Financial state, employment and leisure***

The lowest ratings among the factor groups in our research were allocated to *financial state and leisure time* (see Table 4). With nearly half of the patients being retired and the average income per family member was barely above the state minimum consumer basket value, the financial situation was *completely* satisfactory for 18%, while 30% together were *not at all* or *a little* satisfied with their finances. Older patients put a lot less significance on finances with only 28% of patients ≥75 rating it as a priority, while among those aged ≤44 - 69% rated finances with utmost importance for quality of life. A moderate correlation can be reported for age

and the significance of finances for quality of life ($r$=-0.319; $p$=0.0009), but the sample isn't large enough for the influence to be statistically significant. Other research done in Latvia shows that financial well-being is one of the main drivers of care for health in elderly, but they put much greater value in family ties (20).

**Table 4. Quality of life assessment in the last 2 weeks prior to surgery - Financial state and Leisure time**

| | Not at all | A little | Partially | A lot | Completely | NA |
|---|---|---|---|---|---|---|
| To what extent did your financial situation meet your needs? | 13.3%±5,7% | 16.3%±6,2% | 25.9%±7,4% | 26.7%±7,5% | 17.8%±6,4% | - |
| How available to you was the information you need in your daily life? | - | 3.0%±2,9% | 8.1%±4,6% | 11.1%±5,3% | 73.3%±7,5% | 4.4%±3,5% |
| How much did you enjoy life? | 21.5%±6,9% | 8.9%±4,8% | 18.5%±6,6% | 20.7%±6,8% | 18.5%±6,6% | 11.9%±5,5% |
| To what extent did you have the opportunity for leisure activities? | 29.6%±7,7% | 10.4%±5,1% | 15.6%±6,1% | 20.0%±6,7% | 17.0%±6,3% | 7.4%±4,4% |

(N=135, confidence level 95%)

In our research more than half (58±8%) of the surveyed patients were more or less able to pay for the necessary breast cancer treatment. However among patients aged below 65 years there were 31±10% that weren't able to or found it hard to pay their medical expenses, while among patients 65 and older - 43±11% (a small correlation; $r$=0.178, $p$=0.028). The fact that financial support is needed during treatment at the hospital was noted by 51±8% of patients. Yet for breast cancer patients' treatment and diet requires additional expenses. In many cases a patient's income per capita was just above the poverty level and they did not qualify for financial social support.

*It is a choice between dying or getting treatment, there are only two options. If you work, then you're fine. But if you don't work, and have to survive only on 100 lats, then that's it. It is simply a mockery of people. Realistically with this money I can only cover my utility bills. I have to choose - am I getting treatment or paying my utility bills. (Woman, age 44)*

***State of surroundings and environment***

When considering the four groups of factors, the surveyed patients evaluated most positively questions related to *surroundings and state of environment* with the top two highest values generating over 80% for each section (see Table 5). Of this block of questions the lowest ratings were in relation to the satisfaction of accessibility to health care services (5% *not at all*, 8% *a little*, 10% *partially*).

**Table 5. Quality of life assessment in the last 2 weeks prior to surgery - Surroundings and Environment**

| | Not at all | A little | Partially | A lot | Completely | NA |
|---|---|---|---|---|---|---|
| How satisfied were you with the conditions of your living place? | 1.5% ±2,0% | 2.2%±2,5% | 11.9%±5,5% | 24.4%±7,2% | 56.3%±8,4% | 3.7%±3,2% |
| How safe do you feel in your daily life? | 3.0% ±2,9% | 5.2%±3,7% | 9.6%±5,0% | 25.2%±7,3% | 55.6%±8,4% | 1.5%±2,0% |
| How healthy is your physical environment? | - | 5.9%±4,0% | 5.9%±4,0% | 23.7%±7,2% | 61.5%±8,2% | 3.0%±2,9% |
| How satisfied are you with your access to health services? | 5.2% ±3,7% | 8.1%±4,6% | 10.4%±5,1% | 20.7%±6,8% | 54.1%±8,4% | 1.5%±2,0% |
| How satisfied are you with your access to transportation? | 3.7% ±3,2% | 3.7%±3,2% | 8.9%±4,8% | 14.1%±5,9% | 65.9%±8,0% | 3.7%±3,2% |

(N=135, confidence level 95%)

The quality of health care services was not always satisfactory; patients note not getting enough support from general practitioners (locally known as family doctors) and gynaecologists.

*She isn't a family doctor; I don't know what she is. A family doctor has to be more versatile and that's why she can't have so many patients at one time. She is a pharmaceutical trader, a middleman, that's what she could be. (Woman, 61)*

A research conducted in 2006 showed that respondents were not satisfied with public health care quality and did not trust medical personnel believing that doctors act in their own interest and not the patients (17). Our research shows that patients avoid medical examinations and treatment also due to state of medical facilities and the attitude of medical personnel.

*And I don't like it a little, not a little, but I try to avoid going in. [In the hospital?] Yes, […] if you haven't gone for long, then it's unpleasant. You can feel how it affects your body. […] the space is so narrow, so uncomfortable, it's very depressing. (Woman, 61)*

**Table 6. Quality of life assessment in the last 2 weeks prior to surgery – Quality of Life Factor Importance**

| Rank | Factor | Rating | Not at all | A little | Partially | A lot | Completely | NA |
|---|---|---|---|---|---|---|---|---|
| 1 | Health | 505 | - | - | 1.9% ±2.6% | 5.8% ±4.5% | 92.2% ±5.2% | - |
| 2 | Personal relationships with family and friends | 476 | - | - | 2.9% ±3.2% | 12.6% ±6.4% | 80.6% ±7.6% | 3.9% ±3.7% |
| 3 | Ability to do housework | 459 | - | 1.0% ±1.9% | 8.7% ±5.4% | 19.4% ±7.6% | 68.0% ±9.0% | 2.9% ±3.2% |
| 4 | Financial state | 454 | 1.0% ±1.9% | - | 14.6% ±6.8% | 26.2% ±8.5% | 58.3% ±9.5% | - |
| 5 | Being physically active | 450 | 1.0% ±1.9% | 4.9% ±4.2% | 9.7% ±5.7% | 25.2% ±8.4% | 59.2% ±9.5% | - |
| 6 | Being useful to your family | 446 | 2.9% ±3.2% | 2.9% ±3.2% | 4.9% ±4.2% | 17.5% ±7.3% | 68.0% ±9.0% | 3.9% ±3.7% |
| 7 | Accommodations and environmental conditions | 444 | - | - | 12.6% ±6.4% | 34.0% ±9.1% | 51.5% ±9.7% | 1.9% ±2.6% |
| 8 | Access to health care services | 426 | 1.0% ±1.9% | - | 14.6% ±6.8% | 24.3% ±8.3% | 54.4% ±9.6% | 5.8% ±4.5% |
| 9 | Access to information and media | 404 | 3.9% ±3.7% | 3.9% ±3.7% | 7.8% ±5.2% | 45.6% ±9.6% | 35.0% ±9.2% | 3.9% ±3.7% |
| 10 | Access to transporation | 335 | 2.9% ±3.2% | 5.8% ±4.5% | 18.4% ±7.5% | 26.2% ±8.5% | 30.1% ±8.9% | 16.5% ±7.2% |
| 11 | Physical appearance | 335 | 4.9% ±4.2% | 1.9% ±2.6% | 14.6% ±6.8% | 33.0% ±9.1% | 28.2% ±8.7% | 17.5% ±7.3% |
| 12 | Enertainment and leisure activities | 333 | 10.7% ±6.0% | 6.8% ±4.9% | 23.3% ±8.2% | 37.9% ±9.4% | 15.5% ±7.0% | 5.8% ±4.5% |
| 13 | Abilty to improve yourself | 307 | 25.2% ±8.4% | 3.9% ±3.7% | 10.7% ±6.0% | 24.3% ±8.3% | 27.2% ±8.6% | 8.7% ±5.4% |
| 14 | Ability to do a paid job | 280 | 25.2% ±8.4% | 2.9% ±3.2% | 5.8% ±4.5% | 4.9% ±4.2% | 40.8% ±9.5% | 20.4% ±7.8% |
| 15 | Being useful for society | 268 | 33.0% ±9.1% | 1.9% ±2.6% | 9.7% ±5.7% | 19.4% ±7.6% | 23.3% ±8.2% | 12.6% ±6.4% |

(N=103, confidence level 95%)

## DISCUSSION

Overall the interviewed breast cancer patients rate their quality of life as being average or good at the beginning of treatment. Negative factors contributing to lowered quality of life are mainly linked to patient financial, social and emotional state at the first weeks of treatment and corresponds to previous research done internationally and in Latvia on quality of life issues.

Emotional distress at the start of treatment seems based in the shock of an unexpected diagnosis. Respondents note not being prepared to deal with the idea of a potentially terminal illness and face a lack of emotional and social support from doctors, colleagues and family members. Patient' primary needs are related to the confusion about how breast cancer can develop and how it will affect their lives in short and long term. After first receiving an oncological diagnosis nearly all patients acknowledged suffering from emotional distress that lead to some loss of sleep and fatigue in the 2 weeks prior to surgery. At the early stages of treatment this is the primary issue that can be addressed by medical staff to support patients and improve their quality of life. Moral support should be available from the primary surgeon, family physician and nursing staff, or alternatively patients should be directed to a councillor or support group. There is a need to improve availability of verified and expert approved information about the illness, treatment options and financial support for breast cancer patients.

Finances in general were a problem for the majority of surveyed patients, with only a third being employed and most living on pensions or social support. The added medical expenses were taxing for at least a half of the respondents, yet many were still unaware of what costs to expect from further treatment and what kind of social support is available for cancer patients in Latvia. Over time it could be possible to improve financial support for patients undergoing breast cancer treatment, with prolonged sick leave and treatment coverage, and improve patients' awareness of the illness and diagnosis so that it can be treated at an earlier stage with lower expenses. The improvement of these factors for patients can be addressed at a national and medical facility level. This research was based on a relatively small sample of surveys that were gathered in an ongoing research project. Multifactorial analysis at this point is not statistically viable, but certain trends could be observed and will be tested further once a representative sample of patients has been attained. The topic of *physical appearance* and its influence on breast cancer patient quality of life in particular needs further investigation since many patients avoided talking about this subject having just undergone mastectomy surgery.

## CONCLUSIONS

At the start of treatment breast cancer patient quality of life is influenced primarily by social, emotional and financial factors which should be addressed by medical institution, staff and national policy makers to improve patient treatment strategies and outcomes.

When trying to evaluate and improve different types of treatment for breast cancer patients in Latvia, it seems

important to take into account differences among age groups. Divergent factors influence quality of life for older and younger patients. All age groups place health and personal relationships as priorities for their quality of life, yet when it comes to physical function, outer appearance, finance, leisure and the environment - importance varies. Older patients even prior to surgery were more likely to suffer from physical complaints and were lacking in social and financial support to cope with medical demands and everyday tasks. Younger patients felt greater emotional distress that could lead to biographical disruption.

Data about Latvian breast cancer patient needs affecting quality of life is much too limited at this point. It is particularly important to implement in Latvian hospitals validated quality of life questionnaires that would be carried out with patients of different demographical groups on a regular basis. Further investigation in this area should contribute to the evaluation of breast cancer patients' needs while undergoing treatment and improve the development of effective treatment strategies.

**ACKNOWLEDGEMENTS**

This paper is part of the on-going Riga Stradins University Oncology institute research project "a new multidisciplinary research group formation for the study of breast cancer, therapy effectiveness and patient quality of life improvement" and is financially supported by the European Social Fund (2009/0230/1DP/1.1.1.2.0/09/APIA/VIAA/070).

**Conflict of interest:** None

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10.2478/v10163-012-0002-z



*ORIGINAL ARTICLE*

# Breast – conserving Surgery in Early – Stage Triple – Negative Breast Cancer: Is There a Higher Risk of Locoregional Recurrence?

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**Summary**
**Introduction.** Triple- negative breast cancer (TNBC) is an aggressive disease with poor prognosis and high risk of locoregional recurrence (LRR).
**Aim of the Study.** Is to examine the impact of type of surgery on locoregional recurrence in women with early- stage invasive triple-negative breast cancer (TNBC).
**Materials and Methods.** A total of 68 women with stage I- II (T1N0M0, T2N0M0, T1N1M0, or T2N1M0) invasive, unifocal TNBC with hitologically tumor- free surgical margins were included. Patients were stratified into two groups according to surgical treatment, breast- conserving therapy (BCT) in 36 of 68 patients versus mastectomy in 32 of 68 patients. The two common founder mutations in *BRCA1 (4153delA and 5382insC)* in Latvia were tested using a multiplex- specific polymerase chain reaction(PCR) assay. Clinicopathological data and survival outcomes were analyzed.
**Results.** There were no statistically significant differences in relation to age, stage, tumor size, histological type, tumor grade and nodal status between two groups. 24 patients (77.4%) in the mastectomy group and 27(75%) patients in the BCT group received chemotherapy, these difference was not statistically significant. 10(32.2%) of 32 patients in the mastectomy group and 34(94%) of 36 patients in the BCT group received postoperative radiation (P< 0.0001). There was no statistically significant difference noted in rates of distant metastases (5 cases (16.1%) in the mastectomy group versus 4 cases (11.1%) in the BCT group; P < 0.725)). A higher proportion of patients in the BCT group experienced locoregional recurrence compared with patients in the mastectomy group (3 cases (8.3%) versus 0 case (0%), respectively), but this did not reach statistical significance (P< 0.241). It was found that the tumor histology, grade, age at presentation and *BRCA1* mutation status were not significant predictors of local recurrence. There was no significant difference in 5- year breast cancer- related survival between two groups (P>0.05).
**Conclusions.** Patients after BCT have a higher locoregional recurrence rates compared to mastectomy, but this did not reach statistical significance. According to our study data BCT is not a contraindication in the TNBC.
**Key words:** triple- negative, breast- conserving therapy, locoregional recurrence.

## INTRODUCTION

Triple- negative breast cancer (TNBC) accounts for approximately 15% of all breast cancer subtypes and is characterized by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2). TNBC serves as an immunohistochemical surrogate of basal- like breast cancer subtype, with the accuracy of identification for approximately 80- 97% (7). TNBC is biologically aggressive, BRCA1- related disease associated with poor prognosis, early relapse and shorter survival (1, 2, 4, 7). In the TNBC group distant recurrence risk rises rapidly in the first 3 years after diagnosis. Most distant recurrences and deaths occur within 5 years after diagnosis. There are conflicting data about locoregional recurrence rate in TNBC. Njugen et al. reported significantly higher risk of local recurrence rate in patients with TNBC who underwent BCT versus non- TNBC (adjusted HR 7.1; P= 0.009) (6). In contrast, other groups demonstrated no statistically significant difference in locoregional recurrence between TNBC and other cancer subtypes (3, 5).

## AIM OF THE STUDY

The aim of this study is to examine the impact of type of surgery on locoregional recurrence in women with early- stage invasive TNBC.

## MATERIALS AND METHODS

### Materials

A total of 68 women with stage I- II(T1N0M0, T2N0M0, T1N1M0, or T2N1M0) invasive, unifocal TNBC with hitologically tumor- free surgical margins were included. Patients were stratified into two groups according to surgical treatment, breast- conserving therapy(BCT) in 36 of 68 patients versus mastectomy in 32 of 68 patients. Clinicopathological data and survival outcomes were analyzed.

### Microscopic investigation

Histological parameters of all cases were reviewed by a pathologist. Histological type and grade of ductal breast cancers was determined for each case according to the Bloom-Richardson system. The specimens were selected based on the formalin-fixed, paraffin-embedded breast carcinoma tissue blocks from the primary tumors obtained from the archives of the RAKUS Department

of Pathology and Pauls Stradins Clinical University Hospital. Tumor at surgical margins was considered positive.

**Immunohistochemical analysis**

For this study, triple-negative breast cancers were defined as those that were ER negative, PR negative, and HER2 negative.

ER and PR status were determined using immunochemistry. For ER and PR, monoclonal antibodies were from *DakoCytomation, Glostrup*, Denmark, with cutoff levels for receptor positivity of more than 0%.

The assessment of HER-2/neu expression was carried out using the *HercepTest* kit according to the manufacturer's instructions.

**Genetic testing**

The two common founder mutations in *BRCA1* (4153delA *and 5382insC)* in Latvia were tested using a multiplex- specific polymerase chain reaction (PCR) assay.

**Results interpretation**

ER and PR are considered negative if immunoperoxidase staining of tumor cell nuclei is 0 %. HER2 was assessed through immunohistochemistry (IHC). IHC is scored on a qualitative scale from 0 to 3+, based on interpretation of staining intensity, with 0 and 1+ classified as negative (0- are considered, if staining of tumor cell membrane are less than 10%, and 1+, if more than 10% of tumor cell membrane stains partly ).

The outcomes were analysed in all 68 patients. Follow up has been maintained by reviewing clinical charts. Relapses after 90 days were considered events. Local recurrence was considered as clinical and histological documented relapse in ipsilateral breast or regional lymphnodes.

Distant recurrence was considered as clinical disease distant evidence detected clinically and radiographically.

Statistical analysis: a SPSS statistical software version 12 and Microsoft Excell programm were used for statistical data analysis. Following data were analysed:

1) frequency analysis – were analysed frequencies,
2) descriptive statistics – were analysed minimal, maximal and average value, as well as standart deviation,
3) pair correlation analysis (Spearmans test), having detected p- value and correlation coefficient –r.
4) 5- year breast cancer- related survival- were conducted using the Kaplan- Meier curves.

## RESULTS

Median age at presentation was 59.1 years (range, 27-84 years) in the mastectomy patient group and 53.4 years (range, 31-82 years) in the BCT patient group. There were no statistically significant differences in relation to age, stage, tumor size, histological type, tumor grade and nodal status between two groups (Table 1). 24 patients (77.4%) in the mastectomy group and 27(75%) patients in the BCT group received chemotherapy, these difference was not statistically significant (Table 2). 10(32.2%) of 32 patients in the mastectomy group and 34(94%) of 36 patients in the BCT group received postoperative radiation (P< 0.0001). The median dose to the whole breast was 46 Gy in the mastectomy group and 50 Gy in the BCT group at 1.8- 2.0 Gy/fraction. The median cavity boost in the BCT was 10 Gy at 2 Gy/fraction. 4 patients from 15 tested in the mastectomy patient group were positive for *BRCA1* mutation, compared with 3 patients from 26 tested in the BCT group. The median follow-up period was 42.2 months (range, 9- 84 months). There was no statistically significant difference noted in rates of distant metastases (5 cases (16.1%) in the mastectomy group versus 4 cases (11.1%) in the BCT group; P < 0.725)). All patients with distant metastases in the mastectomy group received previous adjuvant chemotherapy (in 4 cases (80%) - anthracycline- based regimen, in 1 case (20%) - standard CMF). In the BCT group 1 patient (25%) with distant metastases received previous anthracycline- based regimen, 2 patients (50%) - standard CMF regimen and 1 patient (25%) received no chemotherapy. A higher proportion of patients in the BCT group experienced locoregional recurrence compared with patients in the mastectomy group (3 cases (8.3%) versus 0 case (0%), respectively), but this did not reach statistical significance (P< 0.241). It was found that the tumor histology, grade, age at presentation and *BRCA1* mutation status were not significant predictors of local recurrence. All patients in the BCT group who experienced local recurrence had received prior radiotherapy. There were 6(18.7%) deaths in the mastectomy group and 6(16.7%) deaths in the BCT (P=1). There was no significant difference in 5- year breast cancer- related survival between two groups (P>0.05 ) (Fig.1).

## DISCUSSION

To date, a limited number of studies have compared outcomes of TNBC patients after BCT and mastectomy. Parker et al. reported higher local recurrence rate in the mastectomy group versus BCT, but this did not reach statistical significance. The 5-year overall survival, in this study, was better for the BCT group than for the mastectomy group (89% versus 69%; P = 0.018). This was likely due to unsatisfactory balanced groups, the mastectomy group had larger neoplasm size (T3/T4: 4% BCT versus 27% mastectomy; P = 0.0002), advanced N-disease (N2/3: 8% BCT versus 25% mastectomy; P = 0.0003), and advanced stage of disease (stage 3: 8% BCT versus 35% mastectomy; P < 0.0001)(8). According to our study results patients after BCT have shown a tendency of increased risk of LRR compared to mastectomy, but this did not reach statistical significance and had no impact on 5- year breast cancer- related survival. It was found that the tumor histology, grade, age at presentation and *BRCA1* mutation status were not significant predictors of local recurrence. Both groups, in our study, were good balanced with no statistically significant differences in relation to age, stage, tumor size, histological type, tumor grade and nodal status

and number of patients treated with chemotherapy between two groups. All patients in the BCT group who experienced local recurrence had received prior radiotherapy. Although, in the mastectomy group there were a relatively low proportion of patients who received adjuvant radiotherapy, there were no local recurrences during follow- up period (51.8 months, range 9- 84 months).

As with many studies, there some limitations. Although, our study size is relatively small, both groups are good balanced.

Our follow- up period of 3.5 years is relatively short. Dent et al. reported that the risk of any recurrence in the TNBC is high in 1-3 years after diagnosis with majority occuring within the first 5 years(2). Thus, our follow- up period is quite adequate to distinquish the majority of treatment outcomes.

## CONCLUSIONS

Patients after BCT have a higher locoregional recurrence rates compared to mastectomy, but this did not reach statistical significance. According to our study data BCT is not a contraindication in the TNBC.

**Figures and tables.**

**Table 1. Patient and clinical characteristics by operative treatment**

| Variable | BCT (N= 36), n (%) | Mastectomy group (N= 32), n (%) | P- value |
|---|---|---|---|
| Mean age at diagnosis | 53.4 years | 51.8 years | P=0.119 |
| Mean follow-up (m) | 33.8 months | 51.8 months | |
| Tumor size | 21 mm | 23.7 mm | P=0.375 |
| T stage | | | |
| T1 | 19(52.8%) | 13(40.6%) | P=0.739 |
| T2 | 17(47.2%) | 19(59.4%) | |
| Lymph node status | | | |
| N0 | 27(75%) | 19(59.4%) | P=0.394 |
| N1 | 9(25%) | 13(40.6%) | |
| Histology | | | |
| Ductal | 27(75%) | 17(53.1%) | |
| Lobular | 4(11.1%) | 9(28.1%) | P=0.166 |
| Other | 5(13.9%) | 6(18.8%) | |
| Tumor grade | | | |
| I | 2(7.4%) | 1(5.9%) | |
| II | 5(18.5%) | 1(3.1%) | |
| III | 16(59.3%) | 15(88.2%) | P=0.411 |
| Unknown | 4(14.8%) | 0(0%) | |

**Table 2. Characteristics of received chemotherapy in the mastectomy group and BCT group**

| Chara-cteristic | All patients (N= 68), n (%) | BCT (N= 36), n (%) | Maste-ctomy group (N= 32), n (%) | P- value |
|---|---|---|---|---|
| Adjuvant chemo-therapy Anthra-cycline based | 33(48.5%) | 21(58.3%) | 12(37.5%) | P=0.674 |
| Anthra-cycline + Taxane based | 2(2.9%) | 1(2.8%) | 1(3.1%) | |
| CMF | 13(19.2%) | 5(13.9%) | 8(25%) | |
| Cisplatin-based | 2(2.9%) | 0(0%) | 2(6.2%) | |
| No chemo-therapy | 18(26.5%) | 9(25%) | 9(28.2%) | |

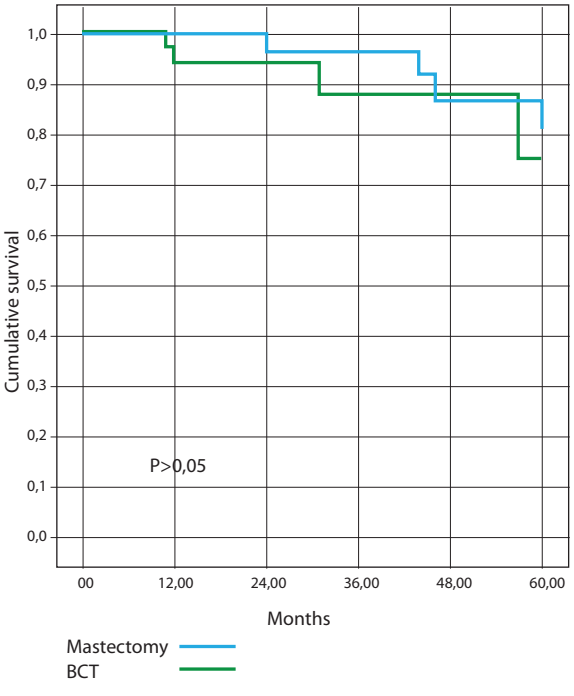


**Fig. 1. Breast cancer- related survival curves of patients after mastectomy and BCT.**

**Conflict of interest:** None

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10.2478/v10163-012-0003-y



*ORIGINAL ARTICLE*

# Unresectable Metastatic Colorectal Cancer Treatment and Survival – Pauls Stradins Clinical University Hospital Experience

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**Summary.**
**Introduction.** Despite recent advances in the medical treatment of metastatic colorectal cancer (mCRC), which include oxaliplatin- and irinotecan-based first-line regimens and the increasing use of targeted monoclonal antibodies, survival rates for patients with mCRC remain unacceptably low.
**Aim of the Study.** Is to analyze survival in patients with unresectable metastatic colorectal cancer.
**Materials and Methods.** Retrospective study of unresectable metastatic colorectal cancer patients who underwent palliative chemotherapy in Clinic of Oncology of PaulsStradins Clinical University Hospital from 2004 to 2011was done.
**Results.**102 patients had a median PFS of 8 months and median OS of 16 months. Subgroup analysis revealed median PFS of 9 months in the synchronous metastatic disease group and 7 months in the metachronous metastatic disease group (p=0.0089) and median OS of 16 months and 12 months, respectively (p=0.0168).Median OS was 11 months in patients received only one line palliative chemotherapy compared to 19 months in patients received more than one line therapy (p<0.0001).
**Conclusions.** The parameter of synchronous and metachronous metastases is of prognostic value in mCRC patients.Second line palliative chemotherapy prolongs overall survival in patient with mCRC.
**Key words:** Metastatic colorectal cancer, palliative chemotherapy, synchronous and metachronous metastatic disease

## INTRODUCTION

Mortality from colon cancer has decreased over the past 30 years, but there is still a huge heterogeneity in survival rates that can be mainly explained by patient and tumor characteristics, host response factors, and treatment modalities. At the time of diagnosis, approximately 25% of the patients already have manifest metastases and almost 50% will develop them following treatment of the colorectal primary [4].

Without treatment, life expectancy is usually < 1 year. The management of unresectable metastatic colorectal cancer (mCRC) is a global treatment strategy, which applies several lines of therapy, salvage surgery and maintenance therapy, in order to prolong overall survival and control symptoms. With modern chemotherapeutic agents, median overall survival (OS) currently reaches 16–22 months [1, 2].

The synchronous presence of primary colon cancer and metastasis may indicate a more disseminated disease status and is associated with a shorter progression free survival (PFS) than metachronous presence of metastasis [3].

## AIM OF THE STUDY

The aim of present study is to characterize survival data in patients with unresectable metastatic colorectal cancer.

## MATERIAL AND METHODS

This retrospective review included 102 patients with metastatic colon and rectal cancer who received palliative chemotherapy at the Pauls Stradins Clinical University Hospital between January 2004 and December 2011. Patients who have had surgery or chemoembolisation for metastatic lesions were excluded. Data were retrieved from each patient's medical records, and included characteristics of the primary and metastatic tumor, chemotherapy information, long-term outcome progression free survival (PFS) and overall survival (OS).Patients were considered evaluable for study if they had completed at least 2 cycles of chemotherapy.

A cut-off value of 6 months after the initial diagnosis to define a metachronous metastatic disease was selected.

Overall survival and progression free survival rates were estimated using the Kaplan-Meier method. The log-rank test was used to calculate any significant difference between the subgroups by univariate analysis. Significance levels were set at $P < 0.05$. All statistical analyses were performed by using MedCalc.

## RESULTS

One hundred and two patients with metastatic colorectal cancer were analyzed. 65 (63.7%) patients demonstrated synchronous metastatic disease, while 37 (36.3%) patients were developed metastases after the primary diagnosis of colorectal cancer.

Patients in the total study group (n=102) had an estimated progression free survival (PFS) of 8 months

and 1 year PFS of 14.6% and estimated overall survival (OS) of 16 months and 1-year OS of 61.8% and 2-year OS of 14.9%.

There were seen increased PFS(p=0.0089; HR 0.45; 95% CI 0.26-0.82) and OS(p=0.0168; HR=0.51; 95% CI 0.30-0.89) in synchronous compared to metachronous metastatic disease group. Median PFS and median OS were 9 and 16 months in the synchronous metastatic disease group, and 7 and 12 months in the metachronous metastatic disease group. The 1-year PFS was 19.1% and 4.7% in the synchronous compared to metachronous metastatic disease group (Fig.1). The 1-year OS was 71% and 44.4% in the synchronous compared to metachronous metastatic disease group and 2-year OS was 18.3% and 0% (Fig.2).

First line palliative chemotherapy received all patients. Oxaliplatin combinations (FOLFOX4, FOLFOX6, mFOLFOX6, oxaliplatin + tegafur) received 72.2%, irinotecan combinations (FOLFIRI, irinotecan mono) – 10.3%, but fluoropyrimidines monotherapy (capecitabine, 5FU, tegafur) – 17.5% of all patients. A small difference in OS and PFS was observed between subgroups (Table 1).

Significant OS difference was observed in metastatic colorectal cancer patients received more than one line of palliative chemotherapy. 50% (n=51) of all patient received second line chemotherapy. 23.53% received fluoropyrimidines, but 76.47% received oxaliplatin and irinotecan containing chemotherapy. Median OS was 11 months in patients received only one line palliative chemotherapy compared to 19 months in patients received combined oxaliplatin or irinotecan containing chemotherapy or fluoropyrimidines(capecitabine, tegafur, 5FU) monotherapy (p<0.0001)(Table 2). 1-year OS and 2-year OS was 80.7% and 26.3% in patients received more than one line of chemotherapy and 40.5% and 0% in patients received only one line of chemotherapy (Fig. 3)

The difference in OS in all patients group between fluoropyrimidines monotherapy and combined cytotoxic treatment was not observed. Median OS was 19 months in both groups (p=0.36) (Fig 3).

Subgroup analysis revealed statistically significant decrease in OS in patients received only one line of palliative chemotherapy in synchronous metastatic disease group. In the metachronous metastatic disease group the difference was observed, but without statistical significance (Table 3).

Targeted therapy impact on survival was analyzed. 13.6% of all patients received one of monoclonal antibodies (bevacizumab or cetuximab) in the first line setting. Difference in PFS or OS was not observed (Table 4).

## DISCUSSION

In this retrospective study we demonstrated our experience in metastatic colorectal cancer treatment.

While surgery is the cornerstone treatment for early stage colorectal cancer, chemotherapy is the first treatment option for metastatic disease when tumor lesions are not resectable at presentation.

Median PFS in first line FOLFOX or FOLFIRI chemotherapy trials reached 8.4 months and median OS 18 months [2]. The addition of bevacizumab or anti-EGFR to oxaliplatin based or irinotecan based first line chemotherapy improve progression free survival to 10.6 months and the overall survival to 21.3 months [8,9]. In patients with *KRAS* wild-type tumors, the addition of cetuximab to either FOLFIRI or FOLFOX produced an improvement in median PFS (9.9 months) and median OS (23.5 months) [10,11]. Similarly, the addition of panitumumab to FOLFOX is superior to FOLFOX alone in terms of PFS [12].

In our study median PFS of 8 months and median OS of 16 months is similar to survival observed in large first line chemotherapy trials. The addition of targeted agents did not show an improvement in median PFS or OS. This fact could be explained due to small number of patients received cetuximab or bevacizumab in the first line palliative treatment.

Only few data have been reported on the prognostic role of synchronous and metachronous metastases in patients with mCRC treated with chemotherapy. Several authors showed no prognostic role for these parameters [2,14,17], whereas others identified metachronous disease as a favorable prognostic parameter [15,16].

There are several studies showing no difference in disease free and overall survival rates between the synchronous and metachronous mCRC after liver resection [5,6].

Current study demonstrated shorter PFS and OS in patients with metachronous mCRC. This finding could be explained with fact that a significant percentage of patients with metachronous metastases were treated with prior adjuvant chemotherapy, whereas patients with synchronous metastases obviously were not. This may have resulted in a resistance to chemotherapy in metachronous mCRC group.

Survival of CRC patients could be influenced by a difference in the presence of prognostic molecular markers between patients with synchronous vs. metachronous metastases [13].

Most patients should be offered second line therapy when tumor progression or unacceptable toxicity stops first line therapy. Our study demonstrated significant overall survival benefit in patient received second line chemotherapy compared to patient who were administered only one line of palliative chemotherapy.

Our knowledge in treatment of unresectable metastatic colorectal cancer is based on clinical trials performed in selected populations, of younger age, and with less co-morbidity than the general metastatic colorectal cancer population. Analysis of current retrospective study included unselected patient population with different treatment strategies and survival outcome.

## CONCLUSIONS

1. Survival data (PFS and OS) is equal to large trial data of first line palliative chemotherapy. Addition of targeted agents in first line settings could improve PFS and OS.
2. The parameter of synchronous and metachronous metastases is of prognostic value in mCRC patients. Possible explanations of decreased survival include a chemotherapy resistance in patients with metachronous disease because of prior adjuvant treatment.
3. Second line palliative chemotherapy prolongs overall survival in patient with mCRC

**Conflict of interest:** None

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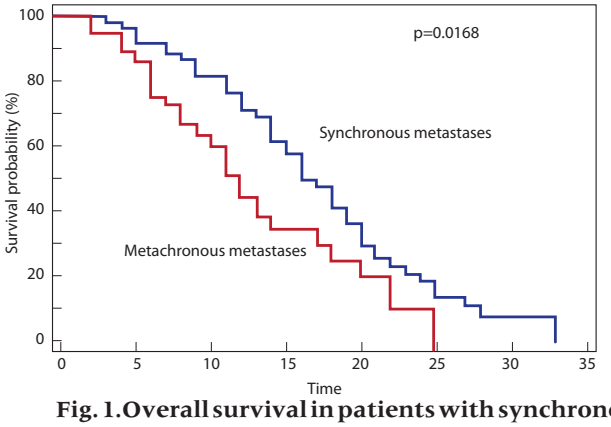


**Fig. 1.Overall survival in patients with synchronous and metachronous metastatic disease.**

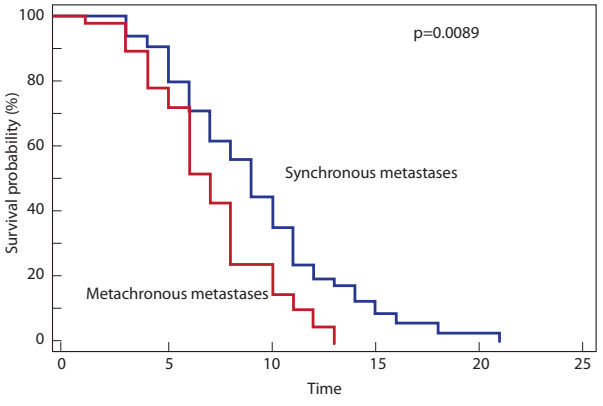


**Fig. 2. Progression free survival in patients with synchronous and metachronous metastatic disease**

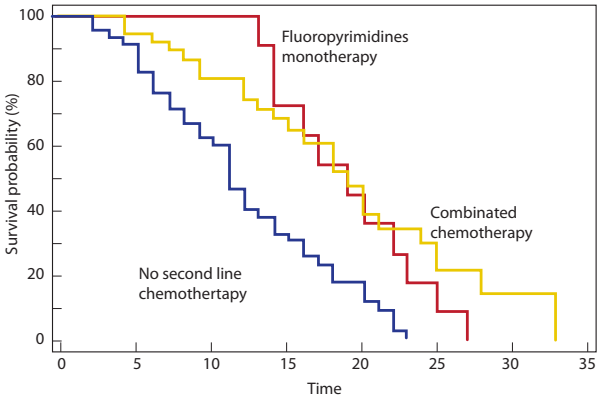


**Fig 3. Overall survival in all patients received second line chemotherapy.**

**Table 1. Median overall survival and progression free survival in patients with synchronous and metachronous metastatic disease received different treatment schedules in the first line settings.**

| | Oxaliplatin containing regiments | Fluoro-pyrimidines mono-therapy | Irinotecan containing regiments | p |
|---|---|---|---|---|
| **Median OS** | **16 months** | **16 months** | **14 months** | **0.47** |
| Synchronous group | 18 months | 16 months | 14 months | 0.26 |
| Meta-chronous group | 12 months | 2 months | 11 months | 0.0007 |
| **Median PFS** | **8 months** | **11 months** | **7 months** | **0.056** |
| Synchronous group | 9 months | 11 months | 7 months | 0.059 |
| Meta-chronous group | 7 months | 2 months | 13 months | 0.6 |

Abbreviations in the table: OS, overall survival; PFS, progression free survival

**Table 2. Median overall survival in patients with synchronous and metachronous metastatic disease received one or more than one line of palliative chemotherapy.**

| | 1 line of chemo-therapy | ≥2 lines of c hemo-therapy | HR | 95% CI | p |
|---|---|---|---|---|---|
| **Median OS** | **11 months** | **19 months** | **0.33** | **0.20-0.56** | **<0.0001** |
| Synchro-nous group | 12 months | 20 months | 0.20 | 0.09774 to 0.4140 | <0.0001 |
| Meta-chronous group | 11 months | 13 months | 0.67 | 0.3041 to 1.4983 | 0.3340 |

Abbreviations in the table: OS, overall survival; HR, hazard ratio; CI, confidence interval

**Table 3. Median overall survival in patients with synchronous and metachronous metastatic disease received one or more than one line of palliative chemotherapy.**

| | No second line therapy | Fluoro-pyrimidines mono-therapy | Combined second line therapy | p |
|---|---|---|---|---|
| **Median OS** | **11 months** | **19 months** | **19 months** | **0.0005** |
| Synchro-nous group | 12 months | 19 months | 20 months | 0.0002 |
| Meta-chronous group | 11 months | 17.5 months | 13 months | 0.57 |

Abbreviations in the table: OS, overall survival

**Table 4. Median overall survival and progression free survival in patients with synchronous and metachronous metastatic disease received chemotherapy (CT) with or without targeted therapy (bevacizumab or cetuximab) in first line palliative settings.**

| | Targeted therapy + CT | CT | HR | 95% CI | p |
|---|---|---|---|---|---|
| **Median OS** | **15 months** | **16 months** | **0.9449** | **0.4516-1.9769** | **0.8803** |
| Meta-chronous group | 11 months | 12 months | 1.1481 | 0.4602-2.8645 | 0.7672 |
| Sync-hronous group | 15 months | 16 months | 1.2159 | 0.3159-4.6796 | 0.7762 |
| **Median PFS** | **9 months** | **8 months** | **0.9413** | **0.4765-1.8593** | **0.8617** |
| Meta-chronous group | 7 months | 7 months | 1.0103 | 0.3847-2.6531 | 0.9834 |
| Sync-hronous group | 10 months | 9 months | 1.1504 | 0.4461-2.9666 | 0.7719 |

Abbreviations in the table: OS, overall survival; PFS, progression free survival; HR, hazard ratio; CI, confidence interval; CT, chemotherapy

10.2478/v10163-012-0004-x

ACTA CHIRURGICA LATVIENSIS • 2012 (12)

*ORIGINAL ARTICLE*

# Surgical Approaches and Postoperative Complcations of Parapharyngeal Space Tumours

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**Summary**
**Introduction.** Parapharyngeal space (PPS) tumors are rare neoplasms that are greatly varying both clinically and morphologically. Complete tumor removal from the PPS requires an integrated approach to resection of nervous and vascular trunks and hemorrhage control.
**Aim of the Study.** This study aimed evaluation of surgical interventions applied for PPS neoplasms along with revision of postoperative complications noticed conducting these surgeries.
**Material and Methods.** 32 PPS tumors removed during surgeries as well as associated biopsies performed at Riga East University Hospital Oncology Center of Latvia at the Department of Head and Neck Surgery from 01.01.2001 till 31.12.2006 were included in this study.
**Results.** The largest number of patients presented with benign salivary gland tumors 22 (68.7%), followed by malignant salivary gland neoplasms 5 (18.5%), neurogenic tumors 4 (12.5%), and miscellaneous tumors 1 (0.3%). Postoperative complications for malignant salivary gland surgeries occurred at the highest rate (42%), whereas, for benign – at the lowest (5%). Transcervical – submandibular, transparotid, and transoral approach was used in 56, 25, and 1% of surgeries, accordingly, whereas, mandibulotomy applied in 17%. "First bite syndrome" was noticed in 30% of the cases, cranial nerve palsies - in 20%, temporary, permanent facial nerve and accessorial nerve injury in 4, 1 and 15% of surgical interventions, accordingly. Vascular trunks damage occurred at 9%, and, mostly, for poststyloid lesions.
**Conclusions.** Heterogeneous PPS tumors show a wide spectrum of postoperative complications and require a selective approach for optimization of surgical intervention and treatment strategy.
**Key words:** parapharyngeal space tumors, transcervical, transparotid and transoral approach, postoperative complications

## INTRODUCTION

Oncologic processes developing within the parapharyngeal space (PPS) lead to appearance of neoplasms subdivided into three categories: salivary gland tumors, neurogenic tumors and miscellaneous tumors. Besides salivary gland primary tumors a direct extension from the deep lobe of parotid gland occurs as well as metastases from elsewhere (9, 11). Clinical manifestations that may direct clinician to a PPS tumor are presented with upper neck or oropharyngeal mass, unilateral Eustachian tube dysfunction, dysphagia, obstructive sleep apnea, cranial nerve deficits, Horner syndrome, pain and trismus (1). Often, however, these tumors even when large present with very little or no symptoms. It may be an incidental finding on an imaging study done for some other reason.

Parapharyngeal space tumors are rare challenging lesions that require a proper understanding of anatomic complexity. The variety of pathologic entities demands a clear understanding of management strategies (5). The goal of parapharyngeal surgery is achievement of complete tumor removal with preservation of integrity of surrounding nerves and vessels and controlling any hemorrhage performed after adequate tumor visualization. Many surgical approaches have been reported in the literature (1, 4, 5, 8, 9) but in overall, transcervical and transparotid approach are the main two. They have also been used with mandibulotomy to increase exposure during surgery (1, 4, 7).

Analysis of surgical interventions performed by Latvian specialists and applied due to removal of heterogeneous tumors developed within the PPS has not been conducted until now. Summarization of data on the surgeries conducted at Riga East University Hospital Oncology Center of Latvia at the Department of Head and Neck Surgery between 01.01.2001 and 31.12.2006, and revision of postoperative complications was accompanied by analysis of head and neck neoplasms affecting the Latvian population. In this study, we analyzed the relative frequency and distribution of the various types of benign and malignant salivary gland tumors, neurogenic tumors, and miscellaneous neoplasms occurring in the PPS.

## AIM OF THE STUDY

In this study we paid a special attention to analysis of surgical interventions conducted using transcervical, transparotid and transoral approach for neoplasm removal and revise complications arising in postoperative period in various types of PPS tumors.

## MATERIALS AND METHODS

32 PPS tumors removed during surgeries as well as associated biopsies performed at Riga East University

Hospital Oncology Center of Latvia at the Department of Head and Neck Surgery from 01.01.2001 till 31.12.2006 were included in this study. Patients were divided into four groups according to the type of PPS pathology: group A (n=22) – benign salivary gland tumors; group B (n=5) – malignant salivary gland tumors; group C (n=4) – neurogenic tumors; group D (n=1) – miscellaneous tumors. Patients were followed–up for three years in order to evaluate the impact of surgical complications on later outcomes. Clinical, laboratory and instrumental data were obtained from medical records. Results were summarized using descriptive statistics methods, and the data on types of PPS tumors, rates and types of postoperative complications were systematized using Microsoft Excel data processing program.

## RESULTS

Group A constituted a majority of tumors 68.7% (22 cases) presented with benign salivary gland neoplasms (Fig. 1). From them, eighty percent were pleomorphic adenomas. In group B constituting 18.5% (5 cases) mucoepidermoid carcinoma was the most common malignant tumor followed by carcinoma ex pleomorphic adenoma. Parapharyngeal neurogenic tumors included in the group C constituted 12.5% (4 cases). These commonly presented with a poststyloid mass, and were subdivided into paragangliomas, carotid body tumors, and vagal paragangliomas or schwannomas. Miscellaneous tumors were rare and heterogeneous neoplasms – 0.3% (1 case). Usually these included lymphomas, hemangiomas, teratomas, lipomas and arteriovenous malformations. The case appeared under the scope of this study was lymphoproliferative disease.
Several surgical approaches to the PPS based on severity of the neoplasm confirmed by histopathologic examination and the exact extend of tumor were used.
The transoral approach was used in 1% of the cases for removal of small, benign neoplasms that originate in the prestyloid PPS and present with an oropharyngeal mass. Limitations of this approach were the restricted access, inability to visualize great vessels, increased risk of facial nerve injury and tumor rupture. The transoral approach may be combined with an external approach to fix lesions with significant oropharyngeal component.
Transcervical – submandibular approach was used in 56% of surgeries. The division of the stylomandibular ligament and retraction of the mandible was used to enlarge the access. Sometimes some pressure through the mouth appeared to be necessary to expel the tumor.
Transparotid - cervical approach was used in 25% of the cases. This approach combined a parotidectomy approach and visualization of the main trunk of the facial nerve and its lower or all branches with a transcervical approach. This method was applied when tumor was attached to or originated from the deep lobe of the parotid gland.
Extended approaches with the mandibulotomy, which gave an excellent exposure to the PPS but was associated with certain morbidity and, therefore, was applied for extensive vascular tumors or recurrent pleomorphic adenoma with multiple large nodules was used in 17% of cases. Various locations for osteotomy have been used including mandibular body, angle, ramus, and parasymphyseal. For lesions localized at the base of the skull an infratemporal fossa approach was needed in 1%.
A rate of complications varied among the groups studied (Fig. 2). The highest rate constituting 45% was demonstrated for B group patients, whereas, the lowest 5% – for A group patients. The spectra and rates of greatly varying postoperative complications occurring in surgical interventions in PPS are summarized at the Figure 3. A common complication in 30% of the cases was so-called "first bite syndrome". Other complications that occurred much less frequently included temporarily facial nerve weakness. The temporary injury of the facial nerve from traction affection and permanent due to inadvertently sacrificing or affected manipulation was appearing in 4 and 1% of surgical interventions, accordingly.
Cranial nerve palsies were demonstrated in 20% of the cases resulted from removal of poststyloid PPS lesions.
Injury of the spinal accessories nerve occurred in 15% of the cases and manifested with weakness of the trapezius muscle, winging of the scapula, and adhesive capsulitis.
Tracheostomy was required for airway protection in 0.5% of the cases when multiple cranial nerve deficits appeared from resection. Palatal weakness, vocal cord paralysis and fistula were fixed very rarely (0.5%).
Cerebrospinal fluid leaks was detected after removal of tumors with jugular foramen or intracranial extension in 1% of the cases. Fascial, adipose or muscular material was used to reinforce the closure.
Vascular complications were commonly associated with removal of neurogenic or vascular lesions. The prevalence of intraoperative vascular injury and of perioperative stroke has been detected at 9% for poststyloid lesions. Avoidance of undue traction on the carotid artery was used for reduction of morbidity, sometimes this pathway resulted in intimal tears. Primary repair or vein grafting was performed for all vessel lacerations.
Injury to the lingual and hypoglossal nerves developed from the transcervical approach when the submandibular triangle was entered was noticed (5%) in addition to the postoperative complications discussed above. Isolated hypoglossal nerve damage did not usually significantly impair swallowing or speech function.
The vagus nerve was the most commonly affected nerve. This injury presented with a vocal cord paralysis, and if injury occurred above the level of the nodose ganglion, laryngeal sensation was also affected.
Complications of mandibulotomy included infection, temporomandibular joint dysfunction, nonunion, plate extrusion, and tooth loss in 5% of the cases. When the osteotomy site was through dental sockets rather than between them, tooth loss was more common.
The recurrence rate of benign PPS neoplasms following surgical extirpation constituted 9%.

## DISCUSSION

Primary parapharyngeal tumors are rare and these are located in a complex anatomical region. Clinical presentation of these tumors can be subtle. Parapharyngeal salivary gland tumors are the most common form of PPS neoplasms, and occur in the prestyloids compartment. Surgical resection is the mainstay of treatment. Transcervial-transparotid approach with or without mandibulotomy is preferred by most surgeons. The transoral is not the approach of choice for most lesions of the PPS (13).

The "first bite syndrome" is the most common complication arising from the damage of the sympathetic innervation of the parotid gland. The myoepithelial cells of the parotid gland may cause supramaximal contraction during the first bite of a meal and give the patient excruciating pain. It has been shown very recently in the paper published by Costa T.P. et al. (2012) that botulin toxin type A has been suggested as a treatment (6).

According to the literature, the risk of postoperative cranial nerve deficits ranges from 11-57% (7, 10, 11), with higher frequencies observed in studies of patients with proportionately greater numbers of malignancies or neurogenic lesions. An isolated nerve injury is usually well tolerated in an otherwise healthy patient. Intraoperative transection of the facial nerve is best managed by performing nerve grafting at the time of surgery. Eye protection is required in the postoperative period - insertion of a gold weight can be performed at surgery or postoperatively.

An isolated unilateral vagal injury is usually well tolerated in the otherwise healthy patient, but a high vagal injury or a vagal injury combined with injury to cranial nerve (CN) IX – n.glossopharyngeus and CN XII - n.hypoglossus, may result in significant problems with swallowing and aspiration. Patients should be carefully evaluated prior to the institution of oral feedings, and an alternate method of feeding (nasogastric tube, gastric tube) should be instituted if necessary (6, 7).

Elderly patients and/or patients with multiple CN deficits are expected to have greater difficulty with swallowing, and medialization as well as cricopharyngeal myotomy should be performed concomitant with resection. The advantage to delaying vocal cord medialization procedures is that patients often compensate, and the extent of medialization required can be better assessed after this has occurred (3).

If swallowing rehabilitation is prolonged or unsuccessful, gastrostomy tube placement may be necessary. Patients with difficulty handling oral secretions may require tracheostomy for airway protection.

Injury to the spinal accessory nerve can be managed postoperatively by an active range of motion physical therapy program. According to Khafif A. et al. (2005), when recognized intraoperatively, transection of CN XI is best managed by nerve grafting to achieve some recovery of function (10).

Horner syndrome may result from injury to the cervical sympathetic chain. The resulting anhydrosis is managed symptomatically.

Since primary parapharyngeal tumors are exceedingly rare, only very few large cohort studies are available. Shahab R. et al. (2005), reviewed 114 parapharyngeal tumors treated over 27 years of experience, whereas, the second study revising large clinical cohort is published recently by Sang J. et al., 2011 (12). According to the literature (2), paragangliomas recur in approximately 5% of cases, and, since 10% are multicentric, the risk of developing a second tumor remains. Patients with a familial paraganglioma syndrome have a 35% risk of multicentricity. In addition, patients with paragangliomas who are being treated nonoperatively must be alerted to the risk of malignant degeneration, which constitutes approximately 10% and is usually associated with rapid growth.

Malignant tumors of the PPS have a much higher rate of recurrence - 25-77%, depending on histology, extent of resection, and duration of follow-up (9). Postoperative radiation therapy for PPS malignancies is recommended to prevent recurrences; however, because of the relative scarcity of these lesions, no large series are available to demonstrate a survival benefit (11).

The 5-year and 10 year survival for benign PPS tumor is 100%. For malignancies the 5 year survival was 93%, but fall to 57% at 10 years. This study showed that a patient is highly unlikely to die of a benign PPS tumor, therefore careful consideration of surgical treatment and discussion with the patient are crucial. While surgery is the mainstay of the treatment for parapharyngeal tumor, radiation therapy should be considered in elderly patients with paragangliomas. According to the literature (11), isolated asymptomatic parapharyngeal schwannomas in elderly patients with no nerve deficit should probably be suspected.

Mandibulotomy can be recommended for vascular tumors extending into the superior PPS, solid tumors that are confined to the superior aspect of the PPS and malignant tumors invading the skull base (8, 13, 14).

Preoperative embolization of vascular lesions may decrease intraoperative blood loss and may facilitate dissection of tumors at the skull base by causing retraction. Embolization is recommended for vascular lesions greater than 3 cm in which obvious feeding vessels can be identified on angiography (10).

The use of embolization of paragangliomas is more controversial. Glomus vagale tumors rarely have a single blood supply, and resection is not associated with excessive blood loss. Embolization of a carotid body tumor may cause an inflammatory response that may obscure the subadventitial plane in which the tumor is dissected, increasing the risk of inadvertent carotid injury during resection. Embolization carries a risk of possible disruption of the blood supply to cranial nerves. If embolization is performed, it is performed within 24 hours prior to resection. Costa T.P. et al. (2012) publication showed that factors as age and multiplicity may also come into the equation when neurologic injury is to be expected. For some patients observation may be the best option, even though the tumor might be technically respectable (6).

It is important to avoid bilateral cranial nerve paralysis in patients with multiple lesions or who have a family history, especially the vagal and hypoglossal nerves. The arguments in favor of observation are that paragangliomas grow very little per year in the order of 1 mm, and they are almost always benign tumors, therefore, morbidity and mortality is low if tumor left untreated.

## CONCLUSIONS

The present study showed that neoplasms resected from the PPS are benign and malignant salivary gland tumors, neurogenic and miscellaneous tumors. The transcervical, transparotid and transoral approach were used for removal of neoplasms. The practical evidences obtained during surgeries and patients' follow-up as well as revision of postoperative complications of heterogeneous PPS tumors is useful for selection of surgical strategy and management of clinical course of tumor and outcome of disease when factors such as anatomical regional complexity, severity and recurrence of neoplasm, age, and other parameters should be taken into consideration.

**Conflict of interest:** None

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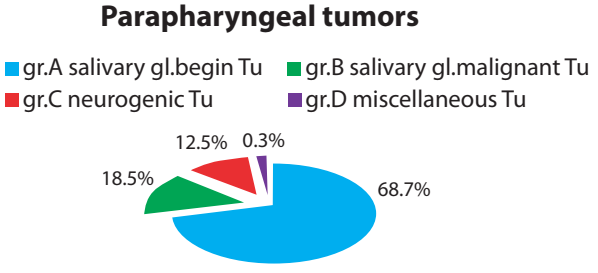


**Fig. 1. Spectra and rates of PPS tumors.**

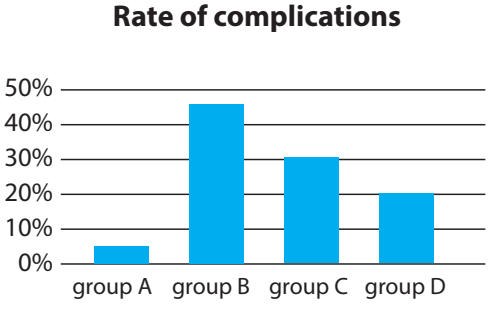


**Fig. 2. Rates of postoperative complications occurring after surgical removal of PPS tumors.**

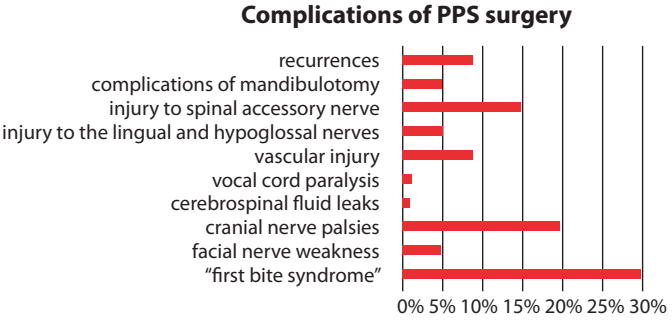


**Fig. 3. Spectra of postoperative complications in PPS surgery.**

10.2478/v10163-012-0005-9

ACTA CHIRURGICA LATVIENSIS • 2012 (12)

*ORIGINAL ARTICLE*

# The Value of Magnetic Resonance in Differentiation between Brain Glioma and Treatment Induced Injury

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**Summary**
**Introduction.** The further therapeutic management decisions in glioma patients after the radiation/chemotherapy may be difficult because the treatment induced brain injury can mimic tumor recurrence clinically and on neuroimaging.
**Aim of the Study** was to assess the usefulness of magnetic resonance spectroscopy (MRS) and diffusion tensor imaging (DTI) in differentiation between glial tumor recurrence and radiation/chemotherapy-induced changes in the brain.
**Material and methods.** 73 patients with primary brain gliomas and 77 gliomas patients after combined therapy with possibly treatment induced changes underwent MRS and DTI. Fractional anisotropy (FA) and metabolite ratios were measured in the tumor and pathological signal intensity area adjacent to post-surgical cavity.
**Results.** Mean choline/creatine (Cho/Cr), myoinositol/creatine (MI/Cr), lactate-lipid/creatine (LL/Cr) ratios of brain gliomas was statistically significant higher and FA values lower than those in the pathological signal intensity area adjacent to post-surgical cavity. No differences were found in mean N-acetyl aspartate/creatine (NAA/Cr) ratios among two groups.
**Conclusions.** Our study suggests that Cho/Cr, MI/Cr, LL/Cr and FA measures should be recommended as additional highly informative tool to conventional structural magnetic resonance imaging (MRI) when monitoring gliomas patients after combined therapy.
**Key words:** brain glioma, treatment induced injury, fractional anisotropy, magnetic resonance spectroscopy.

## INTRODUCTION

Currently, the worldwide recognized treatment method of choice for brain glioma is surgical resection followed by radiotherapy with concurrent chemotherapy (Hygino et al., 2011). The further therapeutic management decisions in glioma patients after the radiation/chemotherapy may be difficult because the treatment induced brain injury can mimic tumor recurrence clinically and on neuroimaging. With the standard magnetic resonance imaging (MRI) sequences differential diagnostic difficulties arise in both contrast enhancing and non-enhancing lesions (Hygino et al., 2011; Brandsma et al., 2008; Nelson, 2011; Sundgren, 2009; Yaman et al., 2011; Weybright et al., 2005). In such cases, new, advanced imaging techniques could be important, which provide physiological and metabolic characteristics of the tumor and surrounding brain tissue (Nelson, 2011). Diffusion tensor imaging (DTI) with fractional anisotropy (FA) quantitative characteristics is non-invasive approach for brain white matter study and particularly in patients with glial cerebral tumors while values of FA reflect the integrity state of white matter tracts (Goebell et al., 2006). Magnetic resonance spectroscopy (MRS) is a technique for non-invasive in vivo assessment of brain metabolites concentration (Bonicelli et al., 2009; Sundgren et al., 2009; Costanzo et al., 2008). It is believed that the MRS may serve as an accurate imaging method for assessment of radiation therapy toxic effects (Weybright et al., 2005; Sundgren et al., 2009). A proposal has been made that combination of DTI and MRS can improve the differentiation of recurrent glioma and post-treatment injury (Nelson, 2011). Metabolites that are observed and well tested in the brain include choline (Cho), creatine (Cr), N-acetylaspartate (NAA), myoinositol (MI), lactate and lipids (LL). NAA is regarded as a marker for neuronal function (Nelson, 2011; Oshiro et al., 2007). Cr is a marker of cellular energy metabolism. Cr is considered the most stable brain metabolite (Sundgren et al., 2009). Cho is a marker of cell membrane (Nelson, 2011; Sundgren, 2009). MI is a marker of glial cells (Kallenberg et al., 2009). Lipids are brain destruction products. Lactate is product of anaerobic glycolysis (Nelson, 2011).

## AIM OF THE STUDY

The aim of this study was to assess the usefulness of MRS and DTI in differentiation between glial tumor recurrence and radiation/chemotherapy-induced changes in the brain.

## MATERIAL AND METHODS

**Patients**. 73 patients (40 women and 33 men, mean age 48 years, range, 14-78 years) with brain gliomas and 77 patients (50 women and 27 men, mean age 48 years, range, 19-72 years) with possible treatment induced injury were studied retrospectively. The tumor diagnosis was determined based on morphological confirmation. Histological type was classified according to the WHO brain tumor classification (Louis et al., 2007) and consisted of 44 glioblastomas, 9 anaplastic oligoastrocytomas, 12 anaplastic astrocytomas, 3 oligoastrocytomas, 3 astrocytomas, 1 anaplastic oligodendroglioma and 1 oligodendroglioma. Treatment

induced injury was diagnosed, based on structural MRI findings in control examination using the following criteria: long-term stability of the structural MRI or spontaneous regression of lesion (Weybright et al., 2005; Huang et al., 2011). The approvals of local Institutional Review Board of the Riga East Clinical University Hospital and the Ethics Committee of Riga Stradins University before study initiation were obtained.

**MR image acquisition**. MR imaging was performed on a 1.5-T General Electric Signa EXCITE MR unit with 8-channel head coil. Standard conventional brain MRI protocol (T2-weighted, FLAIR, diffusion weighted images, unenhanced and gadolinium-enhanced T1-weighted images) was supplemented with MRS ((8ch) PROBE-2DSI PRESS 144TE) and DTI (TENSOR 25 directions 1000b). MRS was performed with multivoxel technique prior to contrast administration. The volume of interest for MRS was determined by using T2-weighted or FLAIR images in axial plane and it was defined including the pathological signal intensity area as well as normal appearing brain tissue. The row data of DTI were obtained using the axial commissural plane.

**MRI data post-processing and image analysis**. Post-processing of MRS and DTI images was performed on a MR GELS (General Electric) workstation. The axial T2 or FLAIR images were used to place defined regions of interest (ROI) in spectroscopic matrix in the tumor as shown in Fig. 1.C, and pathological signal intensity area in the white matter adjacent to post-surgical cavity. We calculated metabolite ratios using the Cr signal as a reference (NAA/Cr, Cho/Cr, LL/Cr and MI/Cr). Round-shaped, uniform sized (30 pixels) ROI for FA measuring was placed in the identical areas (Fig. 2.B).

**Statistical analysis**. Statistical analyses were performed with the Statistical Package for Social Sciences software (SPSS) version 20. We used descriptive statistics to calculate means and standard deviations of measurements. Related sample Wilcoxon signed rank test was used to compare mean metabolite ratios and FA between patients with typical brain glioma and treatment induced injury. P values less than 0.05 were considered statistically significant.

## RESULTS

Results of DTI and MRS were analyzed separately for each group of patients. On the basis of morphological results from biopsy or surgical resection, the lesions of 73 patients were categorized as a glial tumor. On the basis of the clinical and imaging follow-up data the lesions of 77 patients were categorized as radiation injury. The follow-up time of the patients after the initial MRI was a mean of 9.41 months (range, 3–24 months) in patients whose lesions were classified as radiation injury.

Using a nonparametric related sample Wilcoxon signed rank test were compared metabolites and FA values of the tumor and post-treatment injury zone. The mean values (and standard deviations) of the Cho/Cr, MI/Cr, LL/Cr, NAA/Cr ratios and FA in respective lesions are summarized in Table 1. Cho/Cr, MI/Cr, LL/Cr values of glial brain tumors were statistically significant higher and FA values lower than those in the post-treatment zone. No differences were found in NAA/Cr ratios among two groups. Examples of metabolic spectra and FA measurements obtained in areas consistent with glial tumor and treatment induced injury are given in Fig. 1 and Fig. 2.

**Table 1. The comparison of metabolites ratios and FA measurements (mean and standard deviation in parentheses) in the glial tumor and treatment induced injury zone**

| Ratio and FA | Brain glioma (73 patients) | Treatment induced injury (77 patients) | p value |
|---|---|---|---|
| Cho/Cr | 2.305(±1.543) | 1.355(±0.606) | p<0.001 |
| NAA/Cr | 1.031(±0.517) | 1.153(±0.507) | p=0.147 |
| MI/Cr | 0.814(±0.509) | 0.607(±0.362) | p=0.010 |
| LL/Cr | 3.933(±1.547) | 2.304(±1.213) | p<0.001 |
| FA | 0.122(±0.049) | 0.185(±0.065) | p<0.001 |

Abbreviations in the table: Cho, choline; Cr, creatine; NAA, N-acetyl aspartate; MI, myoinositol; LL, lactate and lipid

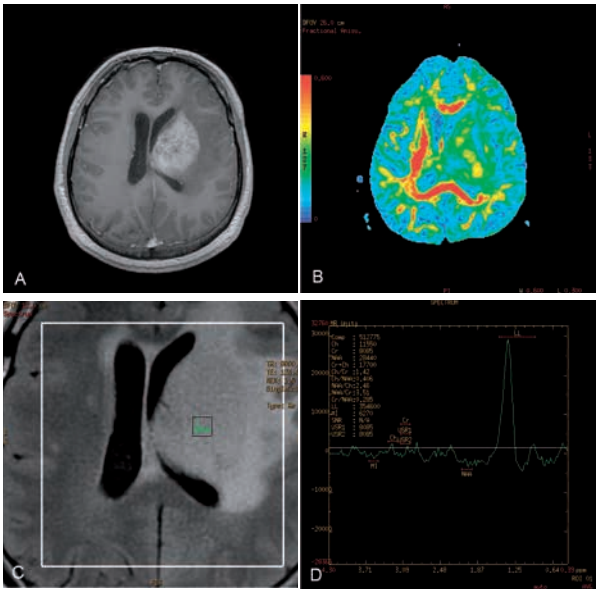


**Fig. 1. MRI in a 45-year-old man with morphologically confirmed recurrent anaplastic oligoastrocytoma. Axial T1 post-contrast MRI shows a heterogeneously enhancing mass in the left frontal lobe. Surrounding hipointensity represents edema and/or tumor cells (A). Axial FA map shows reduced FA in left internal capsule (B). Region of interest for metabolites measurements is placed in the spectroscopic matrix on axial FLAIR image in the tumor (C). Proton MRI spectra from selected region of interest shows high LL peak and reduced NAA peak (D).**

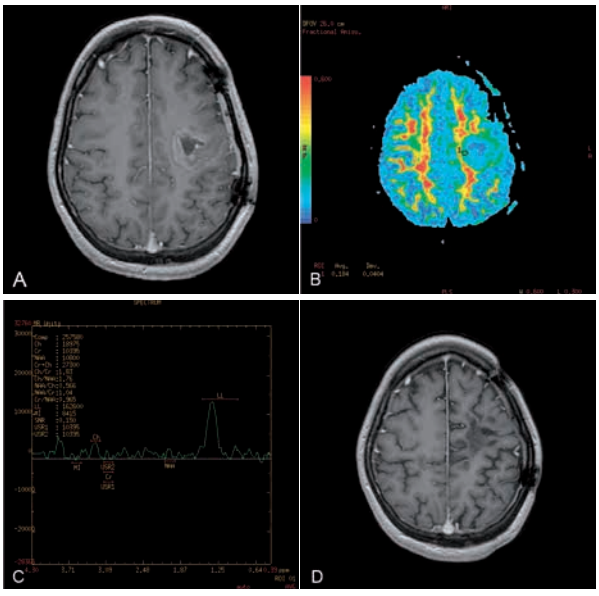


**Fig. 2. 44 year old female with treatment induced injury related to radiation therapy for left parietal anaplastic astrocytoma. Axial post-contrast T1 MRI shows an enhancing lesion around postoperative cavity during radiotherapy 2 months after operation (A). Axial FA map shows reduced FA in left parietal lobe around the resected tumor bed. Region of interest for FA measurement is placed in the pathological signal intensity area (B). Proton MRI spectra from identical selected region of interest shows high LL peak and reduced NAA peak. Compared to the tumor (Fig. 1.D) post-treatment injury shows lower LL peak (C). Axial post-contrast T1 MRI 7 months later shows regression of lesion around post-surgical cavity. No enhancement is seen, typical of late delayed radiation-induced injury (D).**

## DISCUSSION

Routinely, conventional MRI plays an important role in the diagnosis and therapeutic monitoring of malignant brain gliomas. However, interpretation of conventional MRI may be challenging, especially in cases of new contrast enhancing lesion seen adjacent the resected tumor bed (Sundgren, 2009; Yaman et al., 2010; Weybright et al., 2005; Chaskis et al., 2009), because in the post-therapy period the structural MRI is often non-specific – both recurrent and inflammatory and/or necrotic changes due to radiotherapy/chemotherapy typically accumulate contrast (Sundgren, 2009; Principi et al., 2009). Non-enhancing tumors can be difficult to differentiate from other underlying reasons that cause hyperintense changes in T2 and FLAIR images, for example, radiation-induced gliosis (Pope et al., 2011). Temozolamide can promote early radiation damage (Chaskis et al., 2009) that reminds tumor progression (Yaman et al., 2010). Differentiation between radiation necrosis and tumor recurrence is critical for the correct therapeutic management (Nakajima et al., 2009).

This study aimed to evaluate the usefulness of MRS and DTI in differentiating gliomas from therapy induced changes in the brain. We obtained metabolite ratios and FA of brain from 73 patients with brain glioma and 77 patients in clinical and radiological remission with possible treatment induced changes adjacent to previous tumor location.

According to required data, the mean Cho/Cr ratio of glial brain tumors was statistically significantly higher compared with post-treatment injury areas. Achieved results of current study are consistent with the previous report that considers Cho as a marker of tumor cell proliferation (Oshiro et al., 2007). Cho/Cr in recurrent tumor is higher than in the radiation damage (Weybright et al., 2005; Smith et al., 2009). It was observed that Cho/Cr ratio decreases in irradiated brain (Sundgren, 2009; Sundgren et al., 2009).

NAA is a marker of normal brain tissue; it is believed that this indicates the presence of actively functioning neurons (Nelson, 2011). We found no statistically significant difference in mean NAA/Cr ratios between glial tumors and post-treatment injury. The possible morphologic explanation of this fact may be decreased number or loss of neurons in investigated areas. As it is described previously, the neuronal loss and dysfunction (decreased NAA) could be observed in both types of lesions (Hygino et al., 2011). Studies with animal models and human brain autopsy material analysis indicate that the post-treatment injury in the brain include inflammatory changes, demyelination, blood-brain barrier damage and neurotoxic effects (Sundgren, 2009; Sundgren et al., 2009). Some reports have shown that NAA/Cr in recurrent tumor areas is lower than in the radiation damage zones (Weybright et al., 2005; Pope et al., 2011; Smith et al., 2009). Our results, in contrary to above mentioned, agree with opinion that NAA reductions are most probably non-specific indicator, because it occurs at various pathologies (Dincer et al., 2008). NAA/Cr ratio likely reflects the heterogeneity of recurrent glioma with volume averaging of tumor and treatment induced injury or combination of both (Weybright et al., 2005). However, it must be taken into account also that an increase of Cr could also cause a decrease in NAA/Cr (Sundgren et al., 2009).

FA, similar to the NAA, reflects anatomical features of white matter (Goebell et al., 2006). We observed a statistically significantly lower mean FA values in the tumor comparative to altered pericavital zone in patients with clinical remission. These data agree with Sundgren (2009) study, which shows statistically significant higher FA values in normal signal intensity area around the radiation injury compared to normal signal intensity area around the recurrent glioma (Sundgren, 2009).

We observed elevated LL peaks in both groups but the mean LL/Cr ratio in glial tumors was statistically significantly higher compared to those of potential post-treatment injury zones. It is found that lipids increase in apoptosis and necrotic areas that typically observed

in the glial tumor center (Nelson, 2011). Morphologic changes induced by radiation and chemotherapy are also characterized by cell necrosis (Gerstner et al., 1977). Our results suggest that brain destruction is more pronounced in the tumor than in treatment induced injury.

The mean MI/Cr ratio in glial brain tumors in our study was statistically significantly higher compared with those in post-treatment injury zones. To our knowledge, there is no conclusive report on the MI/Cr role in differentiation between glial tumor and treatment induced injury in the literature. Kallenberg and co-authors showed that MI could be increased in a variety of pathologic conditions that involve astrocytic proliferation (Kallenberg et al., 2009).

Several limitations to our study have to be mentioned: First, lack of morphological verification for approval of radiation damage (Huang et al., 2011). Due to the fact that it is an invasive method, it was not performed for patients in remission. Data from the surgical resection and biopsy histological examinations indicate that the radiation damage characteristics of white matter edema, demyelination, fibrinoid changes in blood vessels, coagulative necrosis and cysts (Wang et al., 2010); Second, although regarded as stable metabolite, abnormal Cr levels have been demonstrated also in other different pathologies such as stroke, tumor, and trauma (Sundgren, 2009). In literature there are indications that a reduced level of Cr can be seen in tumors, but the level of Cr may vary in different areas of a tumor (Yerli et al., 2007).

The results of our study show that Cho/Cr, MI/Cr, LL/Cr ratios and FA are different in patients with brain glioma and areas of possible treatment induced injury. According to our data, NAA/Cr ratios do not differ significantly among two investigated groups of patients. We completely agree that MRS results should always be interpreted together with the structural MRI findings, before the final diagnosis (Schillaci et al., 2008).

## CONCLUSIONS

Our study suggests that Cho/Cr, MI/Cr, LL/Cr and FA measures should be recommended as additional highly informative tool to conventional structural MRI when monitoring glial tumor patients after combined therapy.

## ACKNOWLEDGEMENTS

The authors would like to thank Daina Apskalne, PhD, for the carefully conducted morphological analyses. This work was supported by European Social Fund in Latvia (grant number 2009/0147/1DP/1.1.2.1.2/09/IPIA/VIAA/009).

**Conflict of interest:** None

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10.2478/v10163-012-0006-8



*ORIGINAL ARTICLE*

# The Reliability and Accuracy of Knee Implants Sizing Predicted by Digital Templating

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**Summary**
**Introduction.** Osteoarthritis of the knee is a common and frequently symptomatic illness. Total knee replacement (TKR) has evolved as an accepted, cost-effective and efficacious treatment modality for osteoarthritis and other forms of arthritic conditions of the knee joint. Preoperative planning is an important part of the surgical procedure. The inability to accurately determine the magnification factor of the radiograph is one of the major problems in analog preoperative planning of TKR. With the use of calibration objects, the digital images can be corrected for the magnification factor.
**Aim of the Study.** We aimed to determine the reliability and accuracy of digital templating in the pre-operative work-up for TKR.
**Materials and Methods.** A retrospective study was done in 105 caucasian adults, who had osteoarthritis of the knee. Digital templating was performed using a calibrating 25-mm metallic ball and Agfa Orthopaedic Tools digital software package by a surgeon not involved with the operation, who was blinded to the size of the implant inserted. The Press Fit Condylar Sigma Knee system was used in all the patients. Digital anteroposterior and lateral radiographs of the knee were used in measuring the implant size. The results from digital images were compared with the size of actual femoral and tibial implants used at the time of surgery.
**Results.** The correct size of the implant was predicted in 73 of 105 (69,5%) of the femoral and 70 of 105 (66,7%) of the tibial components. The correct size of the whole system was predicted in 58 of 105 (55,2%) cases. The digital preoperative planning predicted 104 of 105 (99,0%) femoral and tibial implants and 103 of 105 (98,1%) whole systems to within one size.
**Conclusions.** We conclude that digital templating using a calibrating 25-mm metallic ball and Agfa Orthopaedic Tools digital software is a reliable method of predicting the implant to within one size.
**Key words:** osteoarthritis of the knee, total knee replacement, digital templating, knee implants sizing

## INTRODUCTION

Osteoarthritis is the result of mechanical and biological events that destabilize the normal processes of degradation and synthesis of articular cartilage chondrocytes, extracellular matrix, and subchondral bone. These changes include increased water content, decreased proteoglycan content, and altered collagen matrix, all leading to the deterioration of articular cartilage. (13)

Osteoarthritis of the knee is a common and frequently symptomatic illness. Its prevalence increases with age, from negligible in those aged 25-34 years to 20-40 per cent in those aged 75 and older. (5)

This number would certainly increase with extended longevity of patients. Severe osteoarthritis of knee joint is a common problem in older people and a major concern for pain and disability. Most patients with osteoarthritis of the knee are able to manage their symptoms with medical treatment and conservative methods, but a large number of patients referred to the specialist surgeon for further management have debilitating disease. (3)

Total joint replacement can be considered as the best end point for clinical trials evaluating disease-modifying osteoarthritis drugs. Great efforts are being made to validate a composite index, which could define states of severity and "need for total joint replacement." (7)

Many parameters other than the severity of the disease itself influence the decision for surgery, however, including socioeconomic factors and access to health services. (6;11;14)

Total knee replacement (TKR) has evolved as an accepted, cost-effective and efficacious treatment modality for osteoarthritis and other forms of arthritic conditions of the knee joint.

Approximately 30,000 TKRs were carried out in 2000/01 in England and Wales (3). The total number of TKRs performed in UK has risen by over 20,000 between the years 2002 and 2004.

The demand for TKR is increasing mainly because of longer life expectancies and rising public expectations for quality of life and mobility in later years. Currently, approximately 2% of the population of 55 years age and above are so disabled that they need TKR, and this rate increases with age. The estimated prevalence in women is nearly twice as high as in men. (15)

TKR rate is increasing not only in UK but also all over the world (Table 1). (1)

TKR is indicated for pain relief and functional improvement in severe knee joint degeneration and arthritis. The goals of TKR surgery include adequate alignment of the prosthesis components and the limb, stability of the knee, and attainment of sufficient range of motion, which permits adequate movement to attain improved quality of life. (4)

Preoperative planning is an important part of the surgical procedure. The technical goals of preoperative

planning of the TKR are to achieve accurate prosthetic seating with proper axial alignment. (10) Preoperative planning provides the surgeon with a tool in order to ascertain that the correct prosthetic component sizes are available. The inability to accurately determine the magnification factor of the radiograph is one of the major problems in analog preoperative planning of TKR. In addition, the use of templates with standard magnifications does not permit accurate correction of the magnification factor. (8)

For the TKR, the analog plans scored poorly concerning exact agreement. Even when allowing for one size difference, the results were disappointing. The digital plans for both components scored better, with more than 50% exact agreements and more than 90% agreements when allowing an error of one component size. The absolute differences between the sizes planned preoperatively for the TKR and implanted component sizes were significantly less for digital planning than for analog planning, regarding both the femoral component (mean difference 0.6; $p < 0.001$) and the tibial component (mean difference 1.1; $p < 0.001$)

With the use of calibration objects, the digital images can be corrected for the magnification factor. This is generally assumed to be an advantage, but if the position of the calibration object differs too much from the region of interest, it will lead to a structural error in digital correction of magnification. In 95% of cases, variability in positioning of the calibration object can be expected to result in an error of correction of the magnification ranging from –3% to +3%. Regarding analog plans for knee prostheses, an actual systematic error in planning seems plausible. (9)

## AIM OF THE STUDY

We aimed to determine the reliability and accuracy of digital templating in the pre-operative work-up for TKR.

## MATERIALS AND METHODS

A retrospective study was done to assess the accuracy of the knee implant sizing predicted by digital images in 105 caucasian adults, who had osteoarthritis of the knee. Digital templating was performed using a calibrating 25-mm metallic ball and Agfa Orthopaedic Tools digital software package by a surgeon not involved with the operation, who was blinded to the size of the implant inserted. The Press Fit Condylar Sigma Knee system was used in all the patients. Digital anteroposterior and lateral radiographs of the knee were used in measuring the implant size. The results from digital images were compared with the size of actual femoral and tibial implants used at the time of surgery.

## RESULTS

The correct size of the implant was predicted in 73 of 105 (69,5%) of the femoral and 70 of 105 (66,7%) of the tibial components. The correct size of the whole system was predicted in 58 of 105 (55,2%) cases. The digital preoperative planning predicted 104 of 105 (99,0%) femoral and tibial implants and 103 of 105 (98,1%) whole systems to within one size. There were 2 cases in which the predicted implant (1 case – femoral, other - tibial) appeared to be undersized from the final component by 2 sizes. The tibial component appeared to be more often undersized– 25 of 105 (23,8%) versa 22 of 105 (20,9%) in femoral component. The rate of femoral and tibial components to be oversized on the preoperative radiographs appeared to be the same - 10 of 105 (9,5%). There were no cases of components to be oversized by 2 sizes.

## DISCUSSION

Not many studies on the reliability and accuracy of knee implants sizing predicted by digital templating can be found up to date and their data is very different.

In this retrospective study we demonstrated our data and tried to compare it with several identical studies. (Table 2)

We can see that the data for exactly predicting femor or tibia size is of a very wide range – from 53,1% to 82,5% in femor and from 59,3% to 79,5% in tibia. In our study digital templating was performed by one of the authors – a surgeon not involved with the operation, who was blinded to the size of the implant inserted. There is no data if the templating surgeon was involved in the operation and thus influencing the choice of the size of the implants in the other studies, so we cannot assume that our result in exactly predicting the implant is really inferior to the one of the third study.

The data for predicting femor or tibia to within one size is very similar in all studies and is not less than 93,0%, which is a very good result. We predicted femor or tibia to within one size in 99,0%, which is the highest result from the studies compared.

There was a trend toward implants to be undersized in digital templating in our study, which can be explained by the will of the templating surgeon to select the implant not overhanging the bone and by the desire of the operating surgeon to preserve as much bone as possible.

Future prospective studies are needed to determine whether preoperative digital templating by the operating surgeon impacts his choice thus improving the accuracy of knee implants sizing.

## CONCLUSIONS

We conclude that digital templating using a calibrating 25-mm metallic ball and Agfa Orthopaedic Tools digital software is a reliable method of predicting the implant to within one size.

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**Table 1. Annualized growth in TKR procedures all over the world**

| Country | Years of available TKR data | Annualized growth in TKR procedures | Annualized growth in procedures rate / $10^5$ |
|---|---|---|---|
| Australia | 2003-2008 | 6,7% | 5,0% |
| Canada | 2002-2008 | 10,3% | 9,1% |
| Finland | 1997-2009 | 7,2% | 6,9% |
| France | 2002-2007 | 5,3% | 3,6% |
| Germany | 2005-2008 | 6,9% | 7,1% |
| Italy | 1999-2008 | 12,8% | 12,2% |
| Netherlands | 1997-2007 | 9,4% | 8,8% |
| Portugal | 1997-2008 | 17,0% | 16,6% |
| Spain | 1997-2008 | 11,5% | 10,1% |
| Switzerland | 1998-2008 | 14,7% | 14,0% |
| USA | 1997-2008 | 7,9% | 6,8% |

**Table 2. Comparison of different studies' data**

| | **Exact femoral size** | **Femor ± 1 size** | **Exact tibial size** | **Tibia ± 1 size** |
|---|---|---|---|---|
| **1st study (16)** | 53,1% | 97,6% | 59,3% | 95,1% |
| **2nd study (12)** | - | 93,0% | - | 93,0% |
| **3rd study (2)** | 82,5% | 97,0% | 79,5% | 92,5% |
| **Our study** | 69,5% | 99,0% | 66,7% | 99,0% |

10.2478/v10163-012-0007-7

ACTA CHIRURGICA LATVIENSIS • 2012 (12)

*ORIGINAL ARTICLE*

# The Management of Extensive Bone Loss in Primary and Revision Total Knee Replacement

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**Summary**
**Introduction.** Total knee replacement (TKR) is still a challenging procedure for severe gonarthrosis patients. Deformities of knee joint (varus, valgus – more than 30 degrees), insufficiency of collateral ligaments and extensive bone loss could be a difficult problem to solve with standard knee endoprosthesis. Also in cases of revision - TKR the restoration of bone loss and regaining of stability of the joint could be a problem.
**Aim of the Study.** The aim of our study was to analyse the results with Rotating - Hinge prosthesis after severe primary and revision TKR.
**Materials and Methods.** 34 patients (27 female, 7 male) were treated with Rotating – Hinge prosthesis during 12 years (first in 1997). Mean age of patients were 69 years. 17 operations were primary total knee arthroplasties and also 17 were revisions of the knee prosthesis. The Oxford Knee score was used for evaluation of patients satisfaction rate. The Knee Society Score was used to get objective functional results. For radiological analysis X-rays of knee joint in two projections were performed.
**Results.** 34 Oxford Knee score questionnaires were sent to patients, response we got from 27 patients (79%). Mean result from Oxford Knee score was 32 which is good. The same number of patients (34) were invited for examination using Knee Society score. The response were from 20 patients (59%). Mean result from Knee Society score was 83, which means excellent.
**Conclusions.** Rotating-Hinge prosthesis allows to achieve good and excellent functional results and high patients satisfaction rate after severe primary and revision TKR. The biological age, general health condition, insufficiency of ligaments and previous infection in patients history have to be considered for choosing the tactics for each case.
**Key words:** severe gonarthrosis, Rotating-Hinge endoprosthesis, semi-constraint.

## INTRODUCTION

TKR is one of the most successful operation in the treatment of gonarthrosis. Inspite of that there are conditions when TKR becomes a challenging procedure. These conditions are severe deformities of knee joint (varus, valgus – more than 30 degrees), insufficiency of collateral ligaments and extensive bone loss. Also in cases of revision - TKR the restoration of bone loss and regaining of stability of the joint could be a problem. Orthopaedic surgeon has a possibility to use standard (non-constraint) endoprostheses and grafting with auto- or allo-bone (to fill the bone defects). For better fixation into the bone endoprostheses with longer stems are available. In cases of severe ligament insufficiency semiconstraint or fully constraint implants could be a solution. In the Riga Hospital of Traumatology and Orthopaedics we used Rotating Hinge (Endo Model) endoprostheses produced by W.Link company (Germany) for the treatment of very severe primary and revision cases.

The Rotating Knee Prosthesis allows axial rotation and reduces the forces acting on the prosthesis anchorage. The prosthesis is semi – constrained, long stemmed, cemented and the material of the prosthesis is made of Co-Cr alloy. Retaining the low friction principle, the physiological movement of the Rotational Knee Prosthesis is optimal because the pivot point is within the physiological area (1; 3-7; 10).

From our point of view the indications to use Rotating Hinge endoprosthesis are - severe deformities of the knee joint as Varus or valgus > 30°, severe insufficiency of collateral ligaments, severe bone loss (especially for patients with low potential for bone healing), insufficiency of muscles (Figure 1).

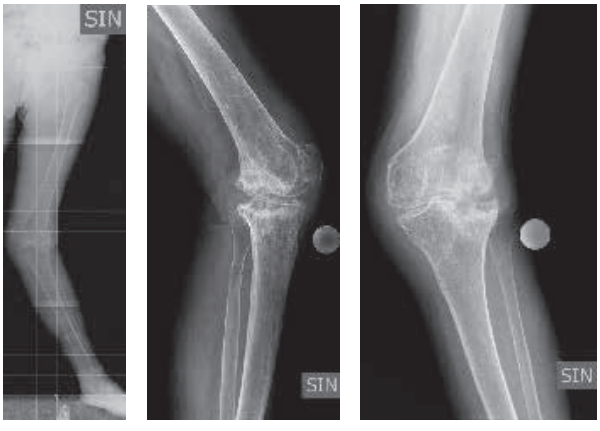


**Fig. 1. Patient with severe gonarthrosis, valgus deformity.**

## AIM OF THE STUDY

The aim of our study was to analyse the results with Rotating - Hinge prosthesis after severe primary and revision TKR.

## MATERIALS AND METHODS

34 patients (27 female, 7 male) were treated with Rotating – Hinge prosthesis during 12 years (first in 1997). Mean age of patients were 69 years. The youngest patient was 45 years old, but oldest – 84 years old. From year 2005 till 2009 32 patients were operated, but in years 1997 and 2003 one patient in each year were operated. 17 operations were primary total knee arthroplasties and also 17 were revisions of the knee prosthesis.

The Oxford Knee score was used for evaluation of patients satisfaction rate. The Knee Society Score was used to get objective functional results. For radiological analysis X-rays of knee joint in two projections were performed.

## RESULTS

34 Oxford Knee score questionnaires were sent to patients, but response we got from 27 patients (79%). Mean result from Oxford Knee score was 32 which is good. 4 were poor, 3 – fair, 13 – good and 7 patients had excellent result (Figure 2). The same number of patients (34) were invited for examination using Knee Society score. The response were from 20 patients (59%). Mean result from Knee Society score was 83, which means excellent. 1 patient had poor result, 2 – fair, 2 – good and 15 had excellent result (Figure 3).

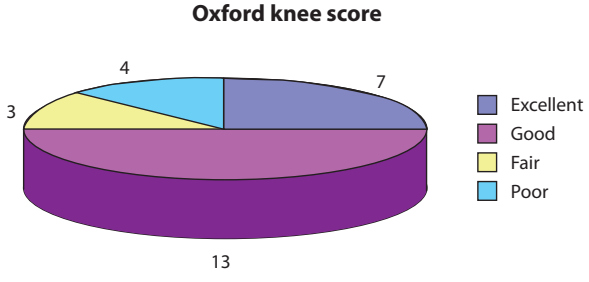


**Fig. 2. The results of Oxford Knee score.**

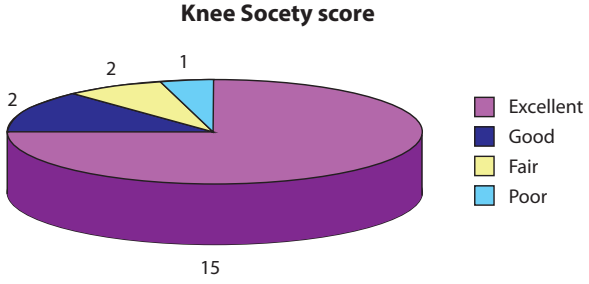


**Fig. 3. The results of Knee Society score.**

There were also 5 complications. 2 deep infections (one - treated by two step revision operation; second - due to critical general condition - amputation), 1 - fracture of patella (3 months after operation – due to severe osteoporosis), 1 - rotational malposition of femural component and 1 periprosthetic fracture of femur (6 months after operation, healed without surgical treatment).

## DISCUSSION

Comparing Oxford Knee Score and Knee Society Score results, we saw that functional outcome was better than patients' satisfaction rate (Figure 4). Our opinion is that Oxford Knee Score results are not always objective because they depend also on the influence of sickness of other joints, side diseases and etc.

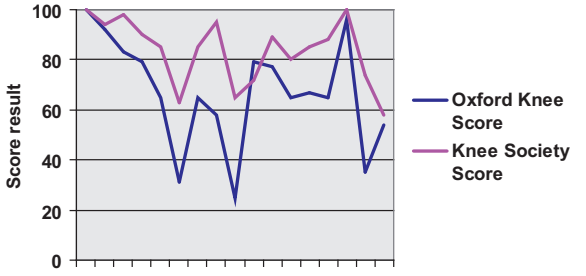


**Fig. 4. Comparison of Oxford Knee Score and Knee Society Score results.**

In the treatment of severe gonarthrosis and difficult revision cases we have to deal with three major problems:

1. Bone defects, mostly segmental.
2. Malalignment
3. Instability due to insufficiency of knee ligaments

There are a two common tactics to manage these problems:

1. Using bone allografts together with non-constraint implants with long cementless stems (cementing only metaphyseal parts of implants);
2. Totally cemented long stem implants with rotating-hinge articular junction.

Some authors (9) propose to perform bone grafting with structural allografts to reconstract the bone defects and restore the joint line. Some of them (9) prefer to use trabecular metal augments. Bone grafting for younger patients with high potential of bone regeneration could be the best choice, but in elder patient group we could deal with graft resorbtion and secondary loosening of implant.

The trabecular metal augments seems to be a solution in those cases, but the huge expenses and absence of long term results let us be precautious in the wider use of this. Our study results show that use of bone cement

even for the substituting of segmental bone defects with additional screw augmentation in older patient group could satisfactory solve the bone defect problem.

To correct a malalignment the authors (8) propose the careful balancing of the flexion and extension gaps with several steps of ligament releases. In severe cases the ligaments are absent or hard damaged that even after release the proper balancing and stable joint is not possible to achieve.

By applying the R-H the dissection of the collateral ligaments leads to the balance in the joint avoiding the asymmetric stress forces on the joint surfaces. The coupling mechanism of rotating hinge reduces the rotational stress forces on the stem – bone, cement –bone interfaces. The so called less constraint implants have about 1° possible rotation between the intercondylar space and polyethylene cam (2). That can lead to the loosening of the stem even if the bone cement fixation is used in methaphyseal segment. In our study we have not faced with aseptic stem loosening with R-H prosthesis.

The use of long stemmed non-constrained implants is definitely useful to heal large bone defects in cases of severe osteoarthritis. From our point of view this method is useful for younger patients. The bone healing potential for older ones is low. The reason why we prefer R-H is that even in very severe cases after TKR with R-H we get stable and moving knee and possibility of partial or full weight – bearing from the second day after operation.

## CONCLUSIONS

Rotating-Hinge prosthesis allows to achieve good and excellent functional results and high patients satisfaction rate after severe primary and revision TKR. The biological age, general health condition, insufficiency of ligaments and previous infection in patients history have to be considered for choosing the tactics for each case.

**Conflict of interest:** None

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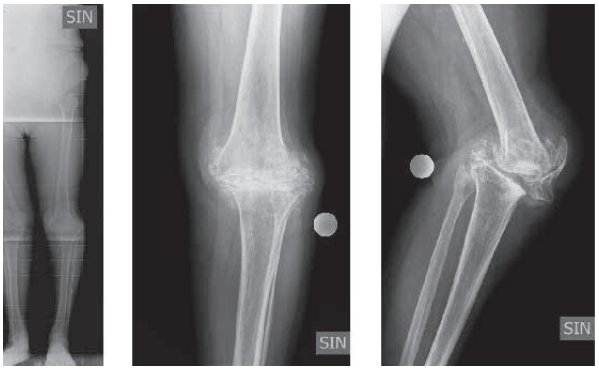
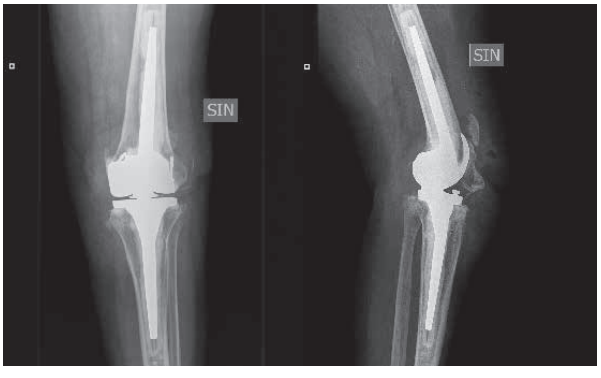
**CLINICAL EXAMPLES**

I. Patient S.K. (female, 72 y.o.)



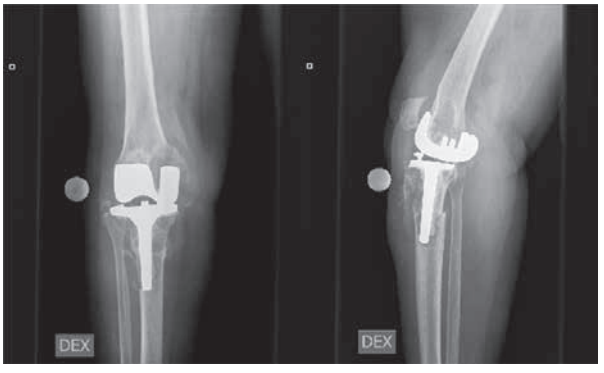

Severe gonarthrosis due to septic arthritis 30 y. ago. Lack of active extension - 60°. (Extensor mechanism damaged during forced flexion of knee 30 y. ago).



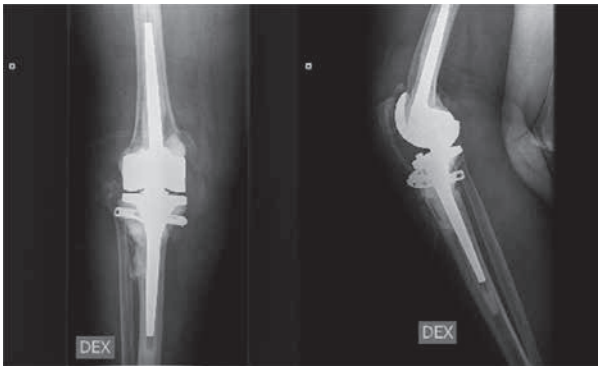

Primary TKR by Rotating-Hinge (Knee was stable, but not full active extension possible).


3 months after primary TKR musculus rectus femoris V-type reconstruction made

II. Patient J.A. (female, 83 y.o.)




Aseptic loosening of both components and periprosthetic fracture of femur and tibia (12 years after primary TKR)




1 stage revision TKR with Rotating-Hinge

Functional result

10.2478/v10163-012-0008-6

ACTA CHIRURGICA LATVIENSIS • 2012 (12)

*ORIGINAL ARTICLE*

# A 5-year Overview of Forearm Fracture Etiology and Treatment Options in 7-15 Years Old Children

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**Summary**
**Introduction.** Forearm fractures make up a significant part of overall fracture rate in pediatric population, especially in 7-15 years old children. Different methods of treatment have been used, depending on the age of children and type and localization of fracture. Most controversies can be seen among conservative and surgical methods of treatment.
**Aim of the Study.** The aim of our study is to identify common localizations and types of forearm bone fractures in pediatric population, as well as analyze patient data and treatment process depending on selected method of treatment for out-patients and in-patients.
**Materials and Methods.** Retrospective analysis of out-patient and in-patient records, treated in University Children's hospital from 2007 to 2011 was made, including first time patients with fractures of one or both forearm bones, according to ICD-10 codes S52.0–S52.9. Demographical data, trauma mechanisms, localization and type of fracture, as well as applied treatment and stay length at hospital were analyzed. 1742 out-patients and 1029 in-patients, 7–15 years old at the moment of trauma, were included in this research.
**Results.** 2771 forearm fractures were registered, 62.9% patients were treated on out-patient basis, 37.1 % patients required treatment in hospital. Forearm bone fractures were gender specific – 2235 boys and 536 girls had to be treated (Male:Female ratio was 4.2 : 1). The peak incidence was seen in 13 years old boys and girls. Boys suffered from forearm fractures more often in any age group. Most common mechanisms of injuries causing forearm fractures in children were related with sports trainings - 22.1%, skiing - 15.0% and traffic injuries - 10.0%. Most common activities at the moment of trauma differ by season – during winter months they include skiing, skating and sledging while in summer falls from height, bicycles and swings are dominant. Several trauma mechanisms, like sport trainings, are not season-dependent. Some injury mechanisms differ significantly by gender. Boys were more often as girls injured during sports trainings and skiing, while girls experience forearm fractures due to bicycling and skating. Occurrence of forearm fractures in children has seasonal differences with two peaks: from June to August and from December to February. Distal forearm fractures are the most often seen localization of overall forearm fractures (42 % in boys and 36 % in girls). In out-patients group conservative treatment was performed – plaster immobilization in 1339 cases and closed reduction, followed by plaster immobilization in 403 cases. In-patients were treated both – conservatively with immobilization in 21 cases and closed reduction in 188 cases, and surgically with K-wire osteosynthesis in 137 cases or elastic stable intramedullary nailing (ESIN) in 683 cases. The type and localization of each fracture, along with the age of patient, are the key factors for choosing the right treatment method. K-wire osteosynthesis was performed in all age groups for unstable fractures in distal or proximal third of forearm. ESIN was a method of choice for unstable or comminuted midshaft fractures of one or both bones, metadiaphyseal fractures and some specific conditions (radial neck fractures, Monteggia fractures-dislocations), especially in older patients. Stay length at hospital was ranging from 1 to 2 hospital days in case of immobilization (mean = 1,05 days), from 1 to 4 days in closed reduction group (mean = 1,32 days), but 1 to 12 days in hospital spent children after K-wire osteosynthesis (mean = 1,99 days) or ESIN (mean = 2,38 days).
**Conclusions.**
1. Forearm fractures in children have a significant gender diversity (M : F ratio is 4,2 : 1).
2. Peak incidence group is 13 years old adolescents of both genders.
3. Seasonality and season-specific injury patterns are typical for pediatric forearm fractures.
4. The most common anatomic localization is the distal segment of forearm bones.
5. Younger children (7–9 years) are mainly treated by conservative methods, while methods of choice for treatment of forearm fractures in adolescents (13–15 years) are operative.
6. Surgical treatment of fractures do not significantly increase stay length at hospital.
**Key words:** Forearm fractures, children, treatment options.

## INTRODUCTION

Child traumatism rates are one of the highest in Latvia if compared to other countries of the European Union. Statistical values do not show signs of improvement year by year (Central Statistical Bureau of Latvian Republic). Forearm trauma is one of the most common traumatic localizations in children, significantly being composed of fractures at different anatomical levels. Incidence of these fractures differs depending on the patient age but the highest levels are shown in 7–15 year old children

(4,5,8,12), therefore this group is analyzed in this research.

Most of the forearm bone fractures are treated conservatively, without invasive intervention; still significant part of patients must undergo surgical stabilization of bone fragments. Modern methods of treatment help us to improve treatment parameters (the amount of intervention, length of treatment, expenses, functional outcomes and quality of life).

Evaluation of forearm fracture parameters may emphasize new details in prophylaxis, showing direction towards reducing trauma prevalence among children. Although forearm fractures are a common pathology in children, there are still significant details of etiology and pathogenesis as well as an optimal selection of treatment methods that underlies this research.

## AIM OF THE STUDY

The aim of this study is to evaluate parameters of forearm bone fractures in elementary school age children (7-15 years old), therefore following objectives were set:

* To identify common localizations and types of forearm bone fractures in pediatric population;
* To analyze patient data and treatment process depending on selected method of treatment in University Children's hospital out-patients and in-patients.

## MATERIALS AND METHODS

Retrospective analysis of out-patient and in-patient records, treated in University Children's hospital from 2007 to 2011 was made, including first time patients with fractures of one or both forearm bones, according to ICD-10 codes S52.0–S52.9. Demographical data, trauma mechanisms, localization and type of fracture, as well as applied treatment and stay length at hospital were the main issues analyzed in this research. 1742 out-patients and 1029 in-patients, 7–15 years old at the moment of trauma, were included in this research. Collected data was generalized, analyzed and compared to foreign research in this area. Descriptive and conclusive statistical methods were used for the study data analysis. $\chi^2$ (chi-square) test was used, based on the p values, to determine the statistical reliability, recognizing the results to be statistically significant if the ratio was less than .05 ($p < .05$). Cross tables and 95% confidence interval were set to compare characteristics of data. SPSS v20.0 and Microsoft Excel software were used for statistical data processing.

## RESULTS

In the five year (from 2007 to 2011) period 2771 forearm fractures were registered in 7-15 years old children. Most of these patients were treated on out-patient basis (n = 1742, or 62.9%; 95% CI 61.0 - 64.7%), still 37.1 % (n = 1029; 95% CI 35.6-39.0%) patients required treatment in hospital. Forearm bone fractures were gender specific – 2235 boys and 536 girls had to be treated (Male:Female ratio was 4.2 : 1). It is obvious that male:female ratio increased with the age.

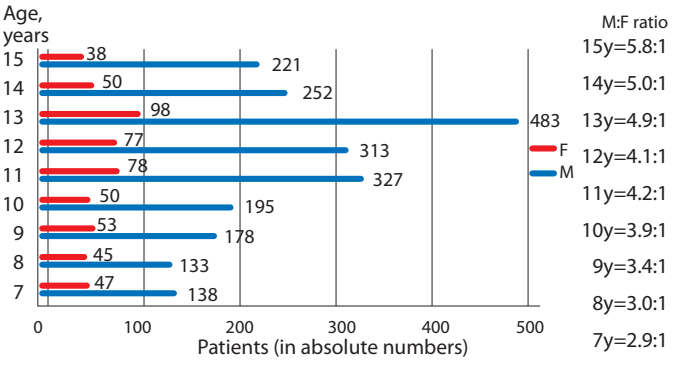


**Fig. 1. Number of patients with forearm fractures by gender and age and male:female ratio by age**

Patients were divided into three age groups: 7-9 years, 10-12 years and 13-15 years old children. The peak incidence was seen in 13 years old boys and girls. Gender differences among age groups were not statistically significant ($p > .05$) – boys suffered from forearm fractures more often in any age group.

Most common mechanisms of injuries causing forearm fractures in children were related with sports trainings (22.1%; 95% CI 20.5 - 23.6%), skiing (15.0%; 95% CI 13.7 - 16.4%) and traffic injuries (10.0%; 95% CI 8.9 - 11.1%) - see Fig.2. Most common activities at the moment of trauma differ by season – during winter months they include skiing, skating and sledging while in summer falls from height, bicycles and swings are dominant. Several trauma mechanisms, like sport trainings, are not season-dependent. Some injury mechanisms differ significantly by gender. Boys were more often as girls injured during sports trainings ($p < 001$; $df=1$; $\chi^2=30.118$) and skiing ($p < .001$; $df=1$; $\chi^2=13.188$) especially in winter months, while girls experience forearm fractures due to bicycling ($p < .001$; $df=1$; $\chi^2=32.916$) and skating ($p < .001$; $df=1$; $\chi^2=31.404$), especially in summer months. Such injury mechanisms, like fall from height ($p=0.059$; $df=1$; $\chi^2=3.566$) and fights ($p=0.206$; $df=1$; $\chi^2=1.596$) do not have statistically significant gender differences.

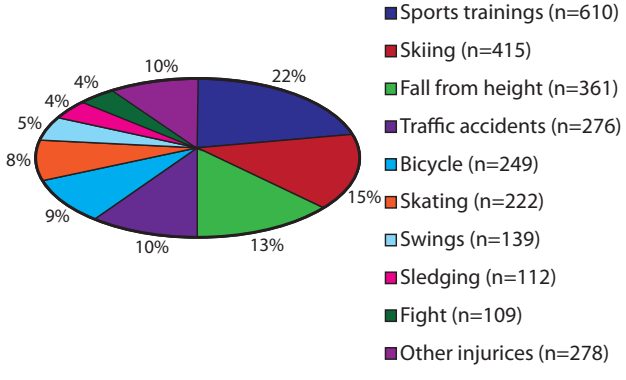


**Fig. 2. Injury mechanisms, causing forearm fractures in children, in absolute numbers and %.**

Occurrence of forearm fractures in children has seasonal differences with two peaks: from June to August and from December to February. Seasonality has gender differences mainly due to the mechanism of injury – boys were more often injured in winter months, while skiing and sledging, but bicycling and roller skating caused more fractures in girls during summer. Seasonality of forearm bone fractures is depicted in Fig. 3.

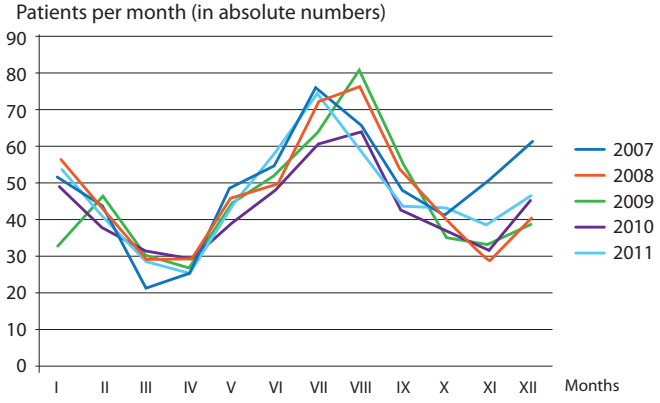


**Fig. 3. Distribution of forearm fractures in children by months of the year (absolute numbers).**

Localization of forearm fractures was classified in accordance to ICD-10 classification codes S52.0-S52.9 and is depicted in Fig.4. It is obvious that distal forearm fractures are the most often seen localization of these fractures (42 % in boys and 36 % in girls). However analyzing statistical significance of gender differences, we can see that neither distal *radius* (S52.5) nor distal *radius* and *ulna* fractures (S52.6) have prevalence in boys (p = .583 and .415 respectively). The same situation is with proximal *ulna* (S52.0) and proximal *radius* (S52.1) fractures (p = .166 and .585, respectively). Opposite tendency can be seen in diaphyseal fractures – in all three groups (S52.2 - *ulna* frx, S52.3 – *radius* frx and S52.4 – both bone frx) boys prevale significantly with p values < .01, < .01 and p = .021, respectively.

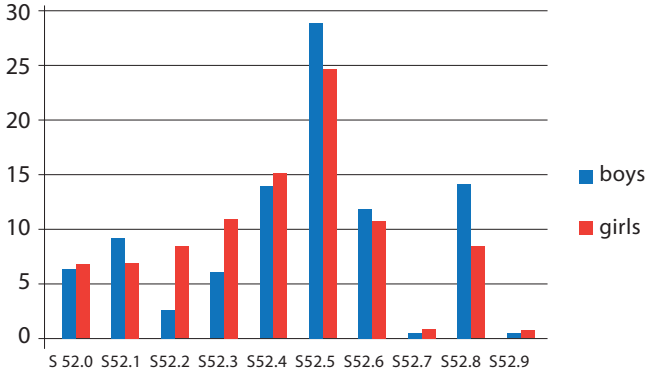


**Fig. 4. Distribution of forearm fractures according to ICD-10 in gender groups (%).**

In out-patients group 2 conservative treatment options were performed – plaster immobilization in 1339 cases and closed reduction, followed by plaster immobilization in 403 cases. In-patients were treated both – conservatively with immobilization in 21 cases and closed reduction in 188 cases, and surgically with K-wire osteosynthesis in 137 cases (percutaneous n = 87, open n = 50) or elastic stable intramedullary nailing (ESIN) in 683 cases (percutaneous n= 616, open n = 67). Overview of treatment methodology is depicted in Fig. 5-1 and Fig. 5-2.

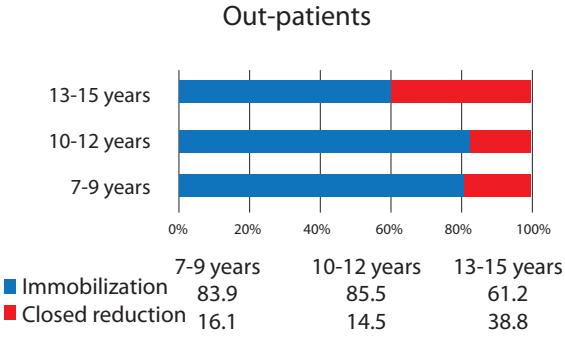


**Fig. 5-1. Out-patient treatment methods of forearm fractures in different age groups (%).**

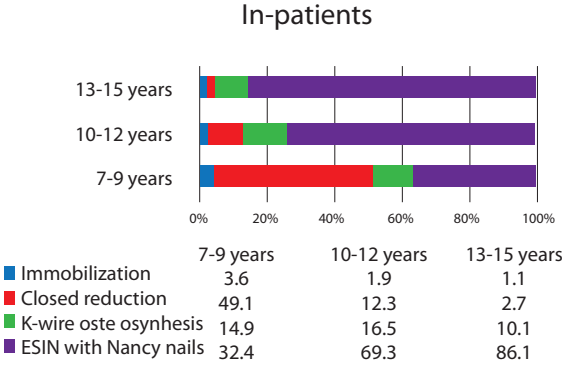


**Fig. 5-2. In-patient treatment methods of forearm fractures in different age groups (%).**

Conservative versus operative methods varied by the age of the in-patients. Half of younger children (7–9 years) were treated by conservative means (immobilization or closed reduction followed by immobilization) – 52.7 % cases (95% CI 46.8-58 %), in 10–12 year age group this proportion was 14.2 % (95% CI 10.8-18.5 %), but 13–15 year old adolescents were treated conservatively only in 3.9 % cases (95% CI 2.4-6.13 %). The type and localization of each fracture, along with the age of patient, are the key factors for choosing the right treatment method. K-wire osteosynthesis was performed in all age groups for unstable fractures in distal or proximal third of forearm. ESIN was a method of choice for unstable or comminuted midshaft fractures of one or both bones, metadiaphyseal fractures and some specific conditions

(radial neck fractures, *Monteggia* fractures-dislocations), especially in older patients.

Stay length at hospital was ranging from 1 to 2 hospital days in case of immobilization (median = 1, mode = 1, mean value = 1,05 days), from 1 to 4 days in closed reduction group (median = 1, mode = 1, mean value = 1,32 days), but 1 to 12 days in hospital spent children after K-wire osteosynthesis (median = 1, mode = 1, mean value = 1,99 days) or ESIN with *Nancy* nails (median = 3, mode = 3, mean value = 2,38 days). Prolonged stay at hospital (> 1 week) was documented in 4 patients after K-wire osteosynthesis (2.9 %) and 14 cases (2.1 %) of patients treated by ESIN.

## DISCUSSION

Forearm bones are very common fracture localization in pediatric population. Several authors mention that almost 50 % of boys and 40 % of girls have experienced at least one fracture episode until their 18 years. Forearm fractures make up 25 % of overall fracture rate in children (4, 8,12).

Data from this survey show that in five year period number of registered patients with forearm fractures does not show tendency to reduce. Of course, data from our hospital reflect the situation in Riga and closer regions only, but taking into account that University Children's Hospital is the only specialized institution in Latvia, and inhabitants of Riga make up approx 1/3 of the whole population (n= 657424, data from Census in 2011), it can be used to reflect situation in the whole country. To achieve more precise result, data from other hospitals should be collected proportionally, in order to reduce data selection error.

Most patients undergo conservative treatment however the number of children treated by surgical intervention due to forearm fractures remains high. Most of the in-patients and out-patients are boys that can be explained by higher activity level in male population. The highest documented levels are in 11-13 years old patients that coincide with other authors (5,7,9).

Patterns of injury mechanism also differ a lot. Most of the injuries during the cold period of year are connected with snow activities – skiing, skating, sledging, etc. Summer period comes with increased numbers of falls from height and bicycle caused injuries. Some of injury patterns, e.g. sports trainings, are not influenced by the seasonal differences. Patterns of injury mechanisms differ in literature, however most of the authors indicate seasonal influence (1,4,8).

Seasonality is seen with two peaks – one is in June-August and corresponds to summer holidays of children and vacation time of their parents, which are usually spent without proper looking after them by nannies or grandparents. The other peak is seen in December-February and can be explained by increased popularity of winter sports that manifests in Latvia during last decade – more and more people involve their children in skiing, skating and other winter activities that inevitably lead to the increase of traumatic injuries. These trauma parameters are high in countries where winter sports are wide-spread and popular (5,10,12).

Analyzing types of fractures, the distal segment seems to be the most common localization for pediatric fractures, followed by diaphyseal fractures. High proportion in distal segment forearm fractures consists of growth plate injuries, that correspond to results of other authors (2,11).

Diagnosis of forearm bone fractures is based on detailed history about circumstances of injury, clinical evaluation of the injured segment and X-rays usually in 2 standard views – AP and LL. If the fracture line is clearly seen, no further investigation is necessary. In case of doubt axial and oblique X-ray views or a CT-scan of injured region can be useful. Ultrasonography or MRI can be used for evaluation of the soft tissue – ligaments, tendons etc. (3,9).

If trauma history and clinical signs of possible fracture are present, yet it cannot be clearly seen on X-rays, especially if involving the growth plate region – one should always keep in mind a possibility of undislocated fracture. These cases substantiate plaster immobilization followed by control X-ray after 7-10 days in which periosteal reaction or other late X-ray signs, showing initial consolidation and confirming diagnosis of fracture, can be seen. Most control X-rays should be performed without plaster as it can contribute to visual artifacts, compromising proper evaluation of them. After confirmation of fracture, immobilization must be restored and kept on the hand for the whole period necessary for full consolidation of the bone fragments (6,10).

All the patients, involved in this survey, underwent treatment on out-patient or in-patient basis. Out-patients were treated by plaster immobilization for undislocated fractures or closed reduction, followed by plaster for dislocated fractures. In-patients consist of two groups. The biggest group is that of surgically treated patients by K-wire osteosynthesis or elastic stable intramedullary nailing (ESIN) with *Nancy* nails. These procedures were performed in percutaneous technique in most cases, only small part of children required open reduction due to anatomically difficult variations of fractures or intraoperative difficulties. The other – smaller group was that of conservatively treated in-patients. This group consisted mainly of younger children, who required general anesthesia for closed reduction.

It is worth mentioning that younger children were mostly treated by conservative means, the proportion of surgically treated children increase with the patient age - from 20.9 % (in 7-9 year patient group), reaching 40.7 % in 13-15 year old patient group. Comparing these parameters across the five year period, we can see overall decrease of operative activity in younger group from 53.4 % in 2007 to 18.3% in 2011 ($p<0.001$; $df=1$; $\chi^2=16.431$), remaining high in 13-15 year old patients (35.2 % in 2007 and 31 % in 2011; $p > .05$). This means that intramedullary stabilization has permanently

become the method of choice for adolescents with midshaft and some specific forearm fractures (radial neck, *Monteggia* fractures-luxations etc.), as it increases stability of bone fractures, reduces immobilization period and complications due to prolonged plaster wearing - joint stiffness and reduction in ROM (6,10). The type and localization of each fracture, along with the age of patient, were the key factors for choosing the right treatment method. Younger children have more capability of remodelation, that allow conservative treatment, immobilizing even fractures with significant displacement, yet achieving almost as good results as after surgical treatment. If surgical treatment is on the issue, one knows that K-wires are excellent for bone end fractures (proximal or distal metaphyses), reserving ESIN with *Nancy* nails for most of unstable diaphyseal fractures, especially in older adolescents. Stable diaphyseal fractures sometimes do not require surgical treatment in any age, so proper immobilization with or without closed reduction, can be used, depending on the amount of dislocation.

Stay length at hospital is rather low for both – K-wire and ESIN patients, ranging from 1-12 days with mean values of 1.99 and 2.38 days, respectively. Prolonged stay at hospital (> 1 week) in 2.9 % of patients after K-wire osteosynthesis and 2.1 % of patients treated by ESIN can be explained by complication occurrence – wound infection, massive hematomas, or pain syndrome, compromising primary healing process.

## CONCLUSIONS

1. Forearm fractures in children have a significant gender diversity (M : F ratio is 4,2 : 1).
2. Peak incidence group is 13 years old adolescents of both genders.
3. Seasonality and season-specific injury patterns are typical for pediatric forearm fractures.
4. The most common anatomic localization is the distal segment of forearm bones.
5. Younger children (7–9 years) are mainly treated by conservative methods, while methods of choice for treatment of forearm fractures in adolescents (13–15 years) are operative.
6. Surgical treatment of fractures do not significantly increase stay length at hospital.

**Conflict of interest:** None

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10.2478/v10163-012-0009-5



*ORIGINAL ARTICLE*

# Radiodensitometric Analysis of Maxillary Sinus-Lift Areas Enforced with Bone Substitute Materials Containing Calcium Phophate

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**Summary**
**Introduction**. An enforcement of atrophic maxilla with biomaterials during sinus-lift surgery improves size and quality of alveolar bone. Radiological densitometric analysis can prove it. Within the study a density of bone was investigated and findings were compared with natural bone around the implants.
**Aim of the Study.** Was to determine if density around the implant inserted with one stage sinus-lift operation using bone substitute materials increase and if that can be detected radiologically.
**Materials and Methods.** Totally there were examined 22 patients where 64 implants were inserted in maxilla: 48 implants were inserted with one stage sinus-lift operation using bone substitute materials; represented study group. Sixteen implants were inserted in natural maxillary alveolar bone of the same patients, representing control group.
The density of implant supporting tissue was measured with cone beam computed tomography and expressed in Hounsfield units (HU). The measurements were performed according to standardized pattern.
**Results.** Densitometric measurements were higher in the study group than in the control group in all points, but statistically significant difference was observed in two measurement sites: buccally in sinus elevation area (BSM) (p=0.005) and palatinally in sinus elevation area (PSM) (p=0.0012), and corresponding areas in control group.
**Conclusions.** Surgical elevation of maxillary sinus floor using calcium phosphate bone substitute materials resulted in higher optical density compared to natural bone. Mineralization of the bone, induced by the bone substitute materials, can be detected radiologically
**Key words:** bone substitute materials, calcium phosphate, sinus-lift operation, cone beam computed tomography.

## INTRODUCTION

There is a huge progress in dental implantology, however, the placement of implants in the posterior atrophic maxilla is still considered to be sophisticated due to reduced bone level (9). Bone reduction in this area occurs due to severe bone loss together with maxillary sinus enlargement observed after teeth extraction. Different solutions, such as: the placement of short, even subperiostal, or long implants in pterygoid process, the autogene bone onlay or/and inlay grafting, tilted implants (2, 3, 11, 14, 17, 18, 22) and maxillary sinus floor elevation (sinus-lift operation) using different grafting materials (4) are proposed to bypass the problem. Usually previous bone transplantation is required. An autologous bone graft is considered to be a 'gold standard' because it is immunocompatible. Autogene bone can be acquired from the donor site intraorally or extraorally (5). As bone transplantation, especially if bone is harvested from extraoral site, is an extensive procedure which not all patients are willing or able to experience, different bone substitute materials are widely used in nowadays. Besides, autografts additionally are associated with donor site`s morbidity and unpredictable resorption of bone graft (19). There are studies revealing native bone grafts do not yield optimal outcome because these bone grafts are tended to remodel around the apex of the implant (21). Calcium phosphate containing bone substitute materials present osteoinductive features and these materials can serve as a pier for a bone growth (1, 19). There are studies where have been proven that artificial bone substitute materials induces bone formation and resorption. Histological and immunohistochemical analysis showed signs of osteoconduction and osteoinduction (6, 10, 19). Also it was shown that hydroxyapatite (HAp), containing bone substitute materials, does not allow taking place the volume changes, as it is with the autogenous bone chips. HAp provides an adequate resistance against an intra-sinus pressure and re-pneumatisation. HAp also is able to maintain a volume of regenerated bone, by formation of a composite network with the biomaterial particles. Such composite bone presents the highest density (15). It has been proven that bone substitute materials are reliable for bone regeneration in sub-antral cavities, and vertical defects observed around the implants are comparable to those observed at implants placed in native bone (16).
Different investigation methods, such as: clinical, radiographs, histo-morphologic, endoscopic, analysis of resonant frequency, biomechanical, etc, have

been applied for evaluation of the results of sinus-lift operation and success of implantations. The radiological method seems to be the least invasive and comfortable for patient. Nevertheless, one should always carry in mind that every radiation exposure to the patient should be justified.
The aim of the study was to determine if density around the implant inserted with one stage sinus-lift operation using bone substitute materials increase and if that can be detected radiologically.

## MATERIALS AND METHODS

Current study was a retrospective radiological study. Totally there were radiologically examined 22 patients. These patients were outpatients that came for implant control during 2010. All patients were in well general health condition. All together there were 64 implants inserted in maxilla. In all patients there were implants inserted in maxillary alveolar bone with both methods: with and without enforcement with calcium phosphate biomaterials. Study group was constructed of 48 implants that were inserted in to the atrophic maxillary alveolar bone with one stage sinus lift operation through lateral window approach with various 1.0 – 2.0 ml calcium phosphate bioceramics granules. Following calcium phosphate biomaterials were used for sinus augmentation operation: Curasan Cerasorb (Germany), Frios Algipore (Germany), Straumann BoneCeramic (Switzerland), RTU Hap granules (LR Patent Nr.P-10-30, Latvia). Control group was constructed of 16 implants that were inserted in to the maxillary alveolar bone of same patients. No biomaterials were used to support and enforce implants in control group. Implants were inserted in usual way as there was enough natural bone to support the implant.
The operation was performed at least seven months before radiological investigation. The longest period between operation and scanning was 7 years. To investigate all sinus lift sites and implants inserted the cone beam computed tomography (CBCT, I-CAT Next Generation, USA) scanning was performed. The cone beam source operated at 120 kV, 5 mA. The scanning parameters were as follows: diameter -16 cm, height - 13 cm, duration - 8.9 seconds. The images of 0.3 voxel size were obtained. Obtained CBCT scans were reformatted and examined using ExamVision software (I-CAT Next Generation, USA). Measurements were done by the sole radiologist. Each implant was aligned into perpendicular position in relation to floor and evaluated in all planes. Measurements were performed in coronal view which passes through the longitudinal axis of the implant.
Density of surrounding tissue around the implant in study group was measured on the following five points (figure 1): density of buccal alveolar bone (BAB), density of buccal bone substitute materials in sinus elevation area (BSM), apically of the implant (AP), density of palatinal bone substitute materials in sinus elevation area (PSM) and density of palatinal alveolar bone (PAB) supporting implant. In control group similar measurements were done in the alveolar bone (figure 1). The measuring field was 0.5 $mm^2$. Measurements were registered in Hounsfield units (HU).
Results were presented as mean and standard deviation (SD). For statistical analysis non–parametric test of significance the Mann-Whitney U test was used for assessment of both groups. Statistical significance was defined as $p<0.05$.

## RESULTS

There were radiologically examined 48 implants placed into atrophic posterior segments of maxillary bone due to surgical sinus floor elevation using bone substitute materials. An average age of study group was 46.7 years, ranging from 32 to 68 years; and it was 48. 8 years in the control group, ranging from 32 to 60. Twenty five implants (52%) were inserted into maxilla of females and 23 implants (48%) were inserted into males.
Reconstruction of the images and alignment of the implants using similar patterns was applied to each patient. It was done for standardization of the method and in order to repeat the procedure.
There was the following mean density around the implants on measuring points in the study group (table 1): buccal alveolar bone - 1018.70 HU ± 387.32, buccal bone substitute materials in sinus elevation area - 953.60 HU ±392.37, apically to the implant - 766.90 HU ± 444.09, palatinally substitute materials in sinus elevation area - 776.60 HU ± 371.61, palatinal alveolar bone supporting implant - 800.60 HU ± 399.02. The mean density around the implants on measuring points in the control group was as follows (table 1): buccal alveolar bone at the crest - 891.00 HU ± 294.00, buccal bone at the apical portion of the implant - 636.80 HU ± 221.03, apically to the implant - 570.10 HU ± 346.77, palatinal bone at apical portion of implant - 482.60 HU ± 215.32, palatinal alveolar bone at the crest - 702.70 HU ± 259.00.
There was found statistically significant difference in density measurements between both groups – p value was 0.005 on buccal bone substitute materials (BSM) in sinus elevation area in the study group, and on buccal bone at apical portion of the implant in control group. Statistically significant difference also was found in density measurements on area of substitute materials of palatinal bone in the region of sinus elevation in the study group, and on buccal bone at the apical portion in control group. Here the p value was 0.0012. No statistically significant differences were found on the other three points of the measurements. The p value for density measurement of BAB was 0.257, for apical tissue density it was 0.13, for palatal alveolar bone 0.28. There was no statistically significant difference regarding radiodensity found among the cases where different biomaterials were used during sinus-lift procedure.

## DISCUSSION

The cone beam computed tomography represents new options for radiological investigation of maxillofacial region. Feasibility to measure density of tissue is one of the options. However, we should understand that the measurements of density in Hounsfield units in CBCT images symbolize a comparative measurement, not a real value of density due to technical specificity.
Control group was represented by the implants inserted

in to the natural maxillar alveolar bone. No bone substitute calcium phosphate biomaterials were used for enforcement of these implants. As that is the basic implant insertions method then these implants were considered the control group.

All sinus lift operations were performed using biomaterials that contain calcium phosphate. In this study comparison of different materials were not curried out as groups were small and the main emphasis was on calcium phosphate that is main component of all biomaterials used in this study. Recent studies in vitro and in vivo showed that such materials provide excellent biocompatibility. In scaffolds of such materials increased number of osteoblasts can be found (23). It is intelligible that a composite of natural bone and bone substitutes, which includes calcium phosphate - after considerable time span, radiologically represents higher density than natural bone alone. Besides, it has been proven in many studies that bone substitute materials used in sinus floor elevation procedures are osteoconductive and osteoinductive, providing good scaffold for new bone formation (6, 9, 10, 19, 21). Measurements around implants in both groups facilitate reproducibility and repeated measurements.

In this study, statistically significant differences of density measurements were found between buccal and palatal sinus-lift sites bone substitute materials areas in the study group, and palatal and buccal bone in the apical portion of the implants in the control group. It represents that mixture of newly formed bone and bone substitute materials around implant construct dense bedding. However, this was not found in apical portion of the implant were also mixture of newly formed bone and bone substitute material was expected. This could be explained that re-pneumatisation of the sinus takes place and this site is more subjected to the remodelling changes, leading to the less material in this area (16). Sinus lift sites when bone substitute materials containing calcium phosphate are used usually remains dimensionally stable for longer period (15). The most dimensional changes occur within first 3 years after operation (7, 8, 12, 24). Several short-time radiological studies demonstrated that majority of resorbtions take place in the first year after sinus lift operation (7, 13). In our study most of implants were examined long after this span of the time, what means - when the most dimensional changes are ceased. Another reason could be the surgical technique, if too little material were inserted in to the apical portion of the implant due to sophisticated operation performed.

There were no statistically significant differences detected between other two measurement areas – palatal alveolar and buccal alveolar bone at the crest in both groups. However, the mean density was higher in the study group. This points the tendency of calcium phosphate bone substitute materials induce mineralization or provides remineralisation of the natural contact bone. There is the study showing that sinus-lift operation when calcium phosphate containing materials were used in long–term decrease augmented area and increase radiodensity in residual, previous demineralised alveolar bone (20).

This study employed small subject groups. To support our results more investigation is required with larger groups.

Conclusion

Surgical elevation of maxillary sinus floor using calcium phosphate bone substitute materials resulted in higher optical density of the mixture of newly formed bone and substitute material around the dental implant as in natural bone alone. Mineralization of the bone, induced by the bone substitute materials, can be detected radiologically.

Wider investigation with larger subject groups is required.

**ACKNOWLEDGEMENTS**

Current study was supported by state research project of Latvia 'New Materials and Technologies for Evaluation and Substitution of Biologic Tissue'.

**Conflict of interest:** None

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**Table 1. Findings of densitometric analysis in the control group and in the study group**

| | Control group (HU) | | Study group (HU) | | p value |
|---|---|---|---|---|---|
| | Mean value | SD | Mean value | SD | |
| 1. BAB | 891.00 | 294.00 | 1018.7 | 387.32 | 0.257 |
| 2. BSM | 636.80 | 221.03 | 953.60 | 392.37 | 0.005* |
| 3. AP | 570.10 | 346.77 | 766.90 | 444.09 | 0.132 |
| 4. PSM | 482.60 | 215.32 | 776.60 | 371.61 | 0.0012* |
| 5. PAB | 702.70 | 259.00 | 800.60 | 388.02 | 0.280 |

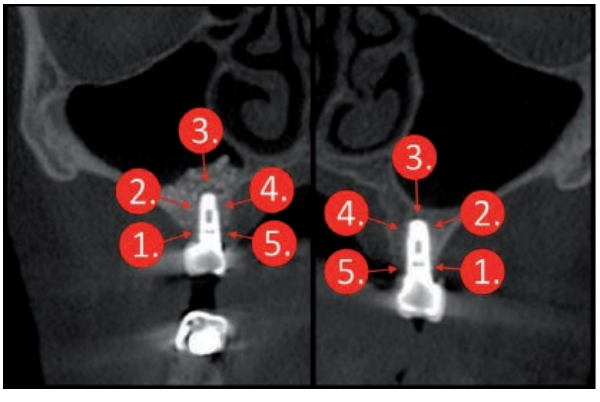


**Fig. 1. Measurements of density around the implant placed into atrophic maxilla with bone substitute materials (left), and around the implant placed into atrophic maxilla without sinus elevation procedure (right)**

10.2478/v10163-012-0010-z



*ORIGINAL ARTICLE*

# Reaction of Subcutaneous Connective Tissue of Experimental Animals on Bone Marrow Mesenchymal Stromal Cell Coated Hydroxyapatite

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**Summary**
**Introduction.** Hydroxyapatite (HAp) scaffolds have become an alternative to autologous bone grafts in orthopedic surgery, because it is possible to fill larger scale bone defects and of the decreased operation time and complication risks at the graft donor site, a promising method in bone regeneration is to load bone marrow - mesenchymal stromal cells (BM-MSCs) on to a HAp scaffold.
**Aim of the Study.** The aim of our study is to compare subcutaneous tissue reaction of experimental animals to implantation of HAp and HAp that is coated with BM-MSCs.
**Materials and Methods.** Autologous BM-MSCs were cultivated from *crista iliaca dextra* biopsy, 4 HAp ceramic implants were coated with BM-MSC and implanted in subcutaneous tissue on one side and non-coated HAp implants on the other side of the spine of 4 rabbits. Immunohistochemical staining of BM-MSC and connective tissue included TUNEL assay, NFkBp105, HSP 70k, Wnt1, TNF-α, VEGF, MMP-2 antibodies, microscoped at 400X magnification and analyzed semiquantitatively.
**Results.** Routine staining with haemotoxilin and eosin demonstrated formation of granulation tissue around the HAp implant, which was more distinct on the control side. There was an increased number of NFκB p105 positive cells (fibroblasts, myocytes, endotheliocytes) on the experimental side (+++), in comparison to the control side (++). HSP70 and apoptosis index was decreased on the experimental side (++ and 60%), in comparison to the control side (+++ and 70%). Number of MMP2 positive structures was increased on the control side in the fibrous capsule (++), in comparison to the experimental side, where the result varied (0 - +)
**Conclusions.** Cultured BM-MSC activity is partly limited due to the apoptosis, which is probably induced by environmental factors. BM-MSC coated HAp implant stimulates secretion of proliferation marker NFkBp105 in subcutaneous tissue, decreases cell stress, apoptosis and tissue degradation (decreased HSP 70, apoptotic cell count and MMP 2) to improve the local tissue quality and proving BM-MSC tissue protective effect.
**Key words:** bone marrow mesenchymal stromal cells, hydroxyapatite, nuclear factor kappa-B

## INTRODUCTION

Hydroxyapatite (HAp) scaffolds have become an alternative to autologous bone grafts in orthopedic surgery, because of the decreased operation time and complication risks at the graft donor site (e.g. *crista iliaca*), such as infection, excessive traumatization and post-operative pain (10). Up to 39% of patients complain of chronic pain at the donor site, other complaints include nerve damage, disturbed gait, hernias and fractures. HAp scaffolds have an advantage over autologous grafts, because it is possible to fill larger scale bone defects with it due to the limited amounts of available autologous graft material (7).

Principal features of biomaterials that could substitute bone grafts, including *osteoconductivity, osteoinductivity and osteogenecity*, were postulated by Marshall Urist in 1965 on the basis of demineralized bone matrix (DBM) (10). *Osteoconductivity* describes two processes: *osteoconversion* (the ability of bone tissue to grow into the scaffold and replace it) and *osteointegration* (the ability of the scaffold to fixate to surrounding bone tissue) (8). HAp is a highly crystalline form of calcium phosphate and is similar to the mineralized phase of the bone. It has a porous structure, which defines the excellent osteoconductive potential of HAp (5, 12). Osteoconduction is a three-dimensional process, which includes vascularization (in-growth of capillaries with perivascular tissue and subsequent migration of osteoprogenitor cell. There is evidence that, when comparing to a control group, where no scaffold is used, use of HAp to fill bone defects improves tissue organization, decreases amounts of fibrous scar tissue by promoting ossification, it also stimulates angiogenesis and allows forming blood vessel shunts through the defect (11). Another study stressed osteoconductivity of HAp *in vivo* in 2.5 year follow-up study on dogs (18). *Osteoinductivity* describes the ability of this biomaterial to stimulate bone tissue formation through various biochemical pathways with certain growth factors and cytokines (4, 7). HAp has a relatively high biocompatibility. In an *in vivo* study on mice inflammatory reaction (proved histologically and by reverse transcriptase Polymerase Chain Reaction (RT-PCR) using IL-1 as an inflammatory marker) to subcutaneous HAp implantation was significantly lower

than to implantation of DBM (10). Several *in vivo* studies state the possibility to utilize HAp as carrier of growth factors to induce ossification, including bone morphogenetic protein – 2 (BMP-2) (5, 19). BMPs induce bone formation and induce the undifferentiated mesenchymal cells to differentiate to osteogenic cells. Other factors that seem to promote bone formation are insulin-like growth factor (IGF-1), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) (4, 12). *Osteogenecity* is obtained if biomaterial scaffold is coated or by any other means has access to osteogenic precursor cells, which subsequently differentiate and form bone tissue (7, 12).

Stems cells are undifferentiated cells that can proliferate and renew its population and have a potential to differentiate into various cell lineages (12). Stem cells in regenerative medicine have been applied to such organs as nerves, blood vessels and heart (17). Mesenchymal stem or stromal cells (MSCs) can be acquired from bone marrow by aspiration with a puncture needle (13, 15, 17). MSCs account for 0.0015 % of subcellular bone marrow component (17), and have been successfully used for bone regeneration in *in vivo* studies on animals and humans (14). MSCs can differentiate into different types of cells, including chondrocytes, myocytes, and osteoblasts (15). One of the main issues with exploiting MSCs in regenerative medicine is the inability to provide these cells with adequate biochemical stimuli, needed to guide the differentiation of MSCs into the necessary lineage, and, therefore, several studies point out the need to add an exogenous soluble growth factor (12, 13). Another issue is the medium, by which MSCs are delivered into tissue so that these cells are sustained at the necessary site (12, 13). A promising method in bone regeneration is to load MSCs on to a HAp scaffold; several studies have demonstrated better results with this method, than when single HAp scaffold was implanted (13, 15, 17). One *in vivo* study on dogs compared HAp coated with MSCs with a control group, where HAp was implanted into the bone defect without the coating. The results demonstrated better vascularization in regenerated tissue in the experimental group. Authors also pointed out that HAp in the control group was encapsulated by fibrotic tissue, which was not observed in the experimental group (17).

## AIM OF THE STUDY

The aim of our study is to compare subcutaneous tissue reaction of experimental animals to implantation of HAp and HAp that is coated with BM-MSCs.\

## MATERIALS AND METHODS

*Implants:*

HAp implants were produced in Riga Technical University Riga Biomaterials Innovation and Development centre. The wet precipitation reaction between calcium hydroxide and phosphoric acid was used to obtain the hydroxyapatite powder. The powder was formed in porous shape and sintered at 1150 °C for two hours.

*Experimental animals and BM-MSCs:*

The Animal Ethics Committee of Latvian Food and Veterinary Administration gave permission to use rabbits for morphofunctional study (Number 24, 02.07.2010). In the study 4 New Zealand male rabbits were included. Biopsy of spongious bone tissue was taken from *crista iliaca dextra* (January 26, 2011) of each rabbit, subsequently this material was grinded and incubated with Type XI collagenase. The cells from this substance were than cultured in an environment containing Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum 20% (FBS) and penicillin/ streptomycin. Cells went through three cell passages, achieving at least 70% confluence of adherent cells. Bone marrow mesenchymal stromal cells (BM-MSCs) were obtained from bone marrow of 4 rabbits. These cells are not proven to be MSCs yet, however they are not haemopoetic cells. Obtained cells were adherent and morphologically similar to MSCs. BM-MSCs were immunohistochemically evaluated in Part I of the experiment that is outlined in the section called *Staining and microscopical evaluation* (***Table 1***).

On February 9, 2011 HAp implants were coated with 100'000 BM-MSCs. The autologous BM-MSCs coated HAp implants were implanted into the subcutaneous tissue of the first 2 rabbits on the right side of the spine – the experimental side. On the left side of the spine – the control side – single HAp implants without BM-MSCs coating were implanted.

On March 8, 2011 euthanasia by air embolisation was performed. Rabbits received general anaesthesia with Ketamini 15 mg/kg and Midazolami 0.5 mg/kg i/m and additional local anaesthesia with Sol. Lidocaini 2% (4 mg/kg). Afterwards specimens of subcutaneus tissue in the region with HAp implants were obtained and immersed into Stefanini solution. These specimens were immunohistochemically evaluated in Part II of the experiment that is outlined in the section called *Staining and microscopical evaluation*.

Similarly, on March 8, 2011 HAp implants coated with 100'000 BM-MSCs and uncoated HAp were implanted on the right and left side of the spine of the remaining 2 rabbits. The specimens for histological analysis were obtained on June 13, 2011. This is outlined in Part III of the experiment.

*Staining and microscopical evaluation:*

The experiment can be divided into 3 parts.

Part I:

Slides containing 20'000 cells were prepared using *Lab-tek II Chamber slide*. Material was stained immunohistochemically with several antibodies:

Nuclear Factor – κB1 p105 (NFkB p105/ p50, 1:100 solution, Abcam, UK)

Heat Shock Protein 70 (HSP 70, 1:100, Abcam, UK)

In order to evaluate apoptosis staining with TUNEL kit (Roche, Germany) was also used.

Part II:

Specimens were stained with haemotoxilin and eosin and immunohistochemically with several types of antibodies: NFkB p105 (NFkB p105/ p50, 1:100 solution, Abcam, UK), TUNEL kit (Roche, Germany),

HSP 70 (HSP 70, 1:100, Abcam, UK) and Wnt1 (1:100 solution, Abcam, UK).

Part III

Specimens were stained with haemotoxilin and eosin and immunohistochemically with several types of antibodies: NFkB p105 (NFkB p105/ p50, 1:100 solution, Abcam, UK), TUNEL kit (Roche, Germany), HSP 70 (HSP 70, 1:100, Abcam, UK) and Wnt1 (1:100 solution, Abcam, UK), tumor necrosis factor-α (TNF-α, 1:100 solution, Abcam, UK), fibroblast growth factor receptor 1 (FGFR1, 1:100 solution, Abcam, UK), vascular endothelial growth factor (VEGF, 1:50 solution, Dako, Denmark), matrix-metalloproteinase 2 (MMP-2 , 1:100 solution, Leica, UK).

All specimens (3 slides for the experimental side and 1 slide for the control side with each staining) were microscoped and photographed at 200x, 250x, 400x magnification and evaluated semi-quantitively. Depending on the relative quantity of positively stained structures, specimens were rated as having a few positive structures (+), moderate quantity of positive structures (++), numerous quantity of positive structures (+++) and abundant amount of positive structures (++++), and a negative result is equal to 0. Apoptosis index was also calculated in specimens stained using TUNEL kit.

## RESULTS

In the Part I of the experiment all bone marrow mesenchymal stromal cells have been apoptotic. BM-MSCs with ameboid structure in the specimens of the first rabbit demonstrated abundant (++++) expression of NFκB p105 - proliferation and inflammatory marker -, however BM-MSCs with a mesenchymal structure demonstrated a negative result (0) for programmed cell death ***Table 2, Figure 1***. Expression of NFκB p105 was numerous (+++) in all BM-MSCs in specimens of the second rabbit. Expression of heat shock protein 70 – indicator of cell stress – varied in specimens of rabbit 1 from negative (0) in cells with mesenchymal structure to numerous (+++) in cells with amebic structure, and was numerous (+++) in specimens of the second rabbit.

In the Part II of the experiment routine staining with haemotoxilin and eosin demonstrated formation of granulation tissue with increased vascularization around the HAp implant, which was more distinct on the control side. There was also inflammatory infiltration on the control side, which was not observed on the experimental side. Apoptosis index (70-90%), NFκB p105 (++++), WNT1 (++++) and HSP70 (++++) positive structures including endothelial cells, fibroblasts, and leukocytes was similar on both sides.

In the Part III of the experiment, routine microscopy demonstrated formation of new muscle fibers, which was more distinguished on the experimental side, some muscle fibers were vacuolised, though no signs of inflammation have been noticed. On the control side rare peri-vascular inflammation can be noticed, dense fibrous capsule has formed around the HAp implant. There was an increased number of NFκB p105 positive cells (fibroblasts, myocytes, endotheliocytes) on the experimental side (+++), in comparison to the control side (++) (***Figure 2 and 3***). HSP70 and apoptosis index was decreased on the experimental side (++ and 60%), in comparison to the control side (+++ and 70%) (***Figure 4 and 5***). Number of MMP2 positive structures was increased on the control side in the fibrous capsule (++), in comparison to the experimental side, where the result varied (0 - +).

## DISCUSSION

All cultured bone marrow mesenchymal stromal cells were apoptotic. This indicates that after the BM-MSCs coated implants are implanted into the subcutaneous tissue of the rabbits, these cells will not differentiate further. We speculate that this might be due to the lack of certain cytokines and growth factors, which normally *in vivo* stimulate osteoblast precursor cells. These factors include bone morphogenetic protein – 2 (BMP2) (6) and IL-3 (1). Several studies also point out the positive role of fibroblast growth factor/ fibroblast growth factor receptor signaling system on the differentiation of osteoprogenitor cells (9). We speculate that these BM-MSCs are capable of inducing proliferation of subcutaneous tissue and regulating inflammatory reactions due to the following reasons.

BM-MSCs in this study stained positive for nuclear factor – kappa B p105/ p50. Several recent studies underline the impact of NF-kB signaling pathway on bone formation through both - osteoclasts and osteoblasts. Inactive NF-kB is located in the cells cytoplasm bound to the inhibitor IkB. When activated, IKK (inhibitor of NF- kB kinase) phosphorylates this complex and thus leads to the liberation and transport of NF-kB p50 to the nucleus, where it regulates expression of genes that enhance inflammatory responses and stimulate proliferation. Tumor necrosis factor alpha (TNF-α) is one of factors that could provoke the NF-kB activation (16). Therefore, it is possible that BM-MSCs may serve as inductors of inflammation and/ or proliferation after BM-MSCs' coated implants are implanted into the subcutaneous tissue.

The NF-kB pathway and its role in regulation of inflammatory response and stimulation of proliferation may explain the lack of distinct fibrous capsule on the experimental side in the III part of our study. The increased levels of NF-kb p105/p50, decreased apoptosis and tissue degradation on the experimental side may be also connected to the inductive stimuli of the BM-MSCs on the HAp implants, even though these cells were apoptotic. This inductive function of BM-MSCs coated HAp implants has been described in several other studies (3, 17). There is evidence that BM-MSCs can stimulate angiogenesis by promoting VEGF expression, this on the other hand is beneficial for bone formation (17). Our experiment did not show any distinct difference of VEGF levels on both sides. There is also information that implantation of BM-MSCs has some immunosuppressive effects (3), this may explain the decreased infiltration and decreased formation of fibrous capsule on the experimental side. One study states that BM-MSCs express cytokines that inhibit apoptosis of surrounding cells (2).

## CONCLUSIONS

1. Cultured BM-MSC activity is partly limited due to the apoptosis, which is probably induced by environmental factors.
2. BM-MSC coated HAp implant stimulates secretion of proliferation marker NFkBp105 in subcutaneous tissue, decreases cell stress, apoptosis and tissue degradation (decreased HSP 70, apoptotic cell count and MMP 2) to improve the local tissue quality and proving BM-MSC tissue protective effect.

**Table 1. Illustrates the algorithm of manipulations held during the course of experiment to obtain specimens of subcutaneous tissue for histological analysis. BM-MSCs coated HAp and uncoated HAp were implanted on the right and left side of the spine in each rabbit.**

| | Part II | | Part III | |
|---|---|---|---|---|
| | Rabbit 1 | Rabbit 2 | Rabbit 3 | Rabbit 4 |
| *Crista iliaca dx* biopsy | January 26, 2011 | | | |
| BM-MSC coated HAp is implanted | February 9, 2011 | | March 8, 2011 | |
| Uncoated HAp is implanted | February 9, 2011 | | March 8, 2011 | |
| Tissue specimens are obtained | March 8, 2011 | | June 13, 2011 | |

**Table 2. Semiquantitative analysis of immunohistochemical BM-MSCs and subcutaneus tissue staining. + - a few positive structures/ week expression, ++ - moderate quantity of positive structures/ moderate expression, +++ - numerous quantity of positive structures/ marked expression, ++++ - abundant quantity of positive structures/ abundant expression, and 0 indicates a negative result.**

| Experiment: | Part I | | Part II | | Part III | |
|---|---|---|---|---|---|---|
| | Rabbit 1 | Rabbit 2 | Experimental side | Control side | Experimental side | Control side |
| TUNEL | 100% | 100% | 80-90% | 70-80% | 60% ↓ | 70% |
| NFκB p105 | 0/++++* | +++ | ++++ | ++++ | +++ ↑ | ++ |
| HSP 70 | 0/+++** | +++ | ++++ | ++++ | ++ ↓ | +++ |
| WNT1 | | | ++++ | ++++ | | |
| Granulations | | | + ↓ | ++ | | |
| FGFR1 | | | | | +++ | +++ |
| VEGF | | | | | 0 - ++++ | +++ |
| MMP2 | | | | | 0/+ | ++ |
| * Mesenchymal cells - 0, ameboid cells - ++++ | | | | | | |
| ** Mesenchymal cells - 0, ameboid cells - +++ | | | | | | |

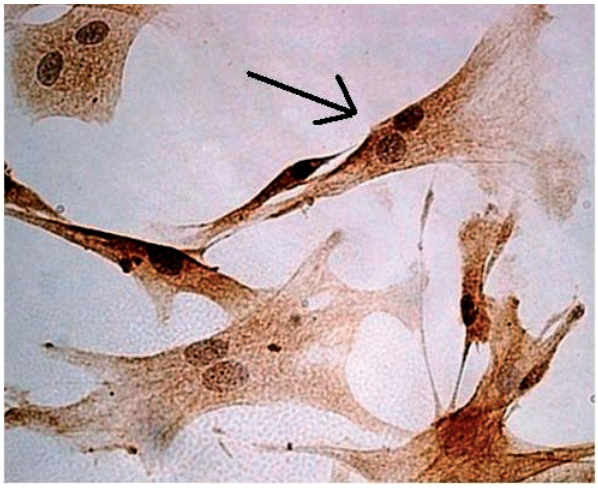

**Fig. 1. NF-kB p105 positive bone marrow mesenchymal stromal cells (black arrow), rabbit I. Immunohistochemistry, anti- NF-kB p105, 400x.**

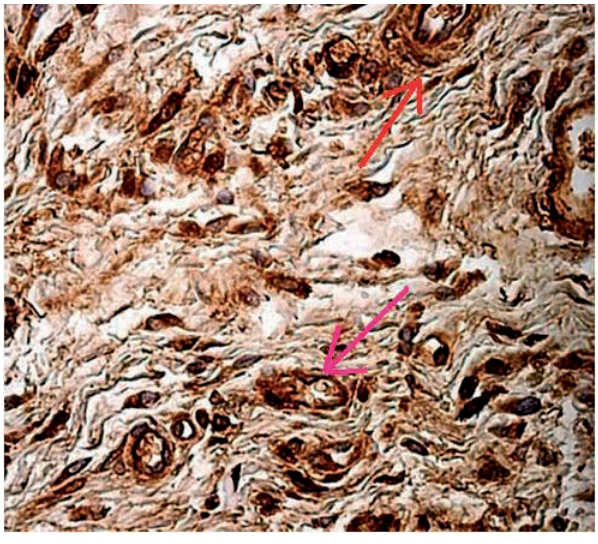

**Fig. 2. NF-kB p105 positive endotheliocytes (pink arrow), myocytes (red arrow), experimental side, rabbit II, Immunohistochemistry, anti- NF-kB p105, 400x.**

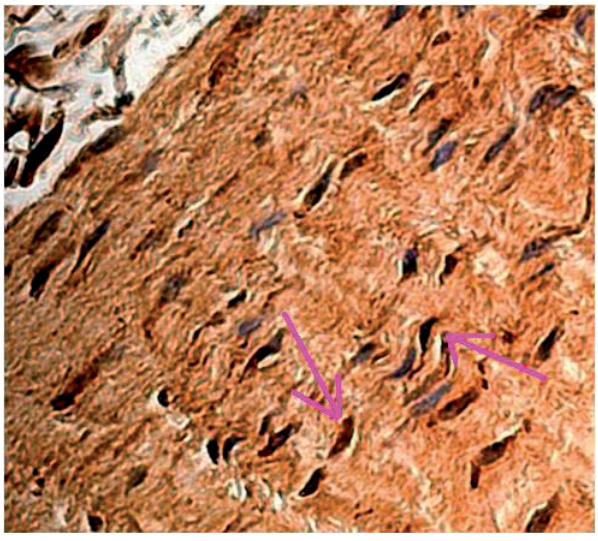

**Fig. 3. NF-kB p105 positive fibroblasts in the fibrous capsule (pink arrows), control side, rabbit II. Immunohistochemistry, anti- NF-kB p105, 400x.**

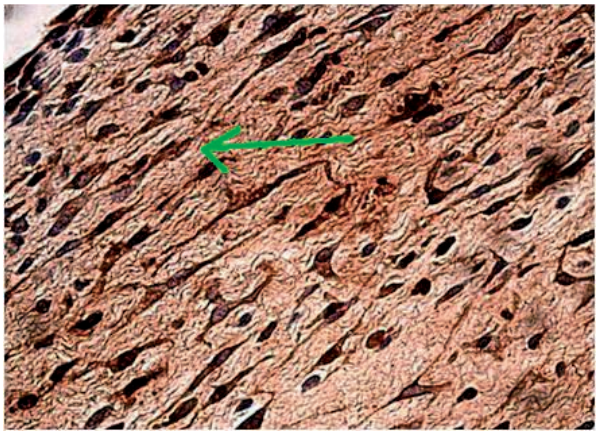

**Fig. 4. Apoptosis of fibroblasts in the fibrous capsule (green arrow), control side, rabbit II, TUNEL, 400x.**

**Fig. 5. Apoptosis of endotheliocytes, experimental side (green arrow), rabbit II, TUNEL, 400x**

**Conflict of interest:** None

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10.2478/v10163-012-0011-y



*ORIGINAL ARTICLE*

# Pulsed Radiofrequency Effects on the Lumbar Dorsal Root Ganglion of the Domestic Porcine: Pilot Study

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**Summary**
**Introduction.** Pulsed radiofrequency (PRF) is a percutaneous minimal invasive procedure for chronic pain management that can be used when conservative treatment methods have been ineffective. The effectiveness of PRF was demonstrated in various good quality randomized control studies, but mechanisms of action are still unclear.
**Aim of the Study.** The aim of our study is to analyse the histological effects of PRF on the domestic porcine dorsal root ganglion (DRG), and evaluate the expression of biomarkers in gangliocytes of the subject(s).
**Materials and Methods.** A total 3 domestic porcines were investigated. Under general anaesthesia and X-ray control, DRG PRF was performed. Four lumbar DRGs ($L_1$, $L_2$, $L_3$, $L_4$) were randomly treated. The opposite side DRGs was used as control. One month after the procedure the animal was euthanized. The lumbar region of the spine was placed in 10% formaldehyde for a month. After this fixation DRG samples were prepared for slide analysis. They were embedded in paraffin in order to obtain 3mm thick sections, which were then cut by microtome and collected on slide glasses. Using standard immunohistochemical reactions, the materials were tinted to define biomarkers neurofilaments (NF), glial fibrillary acidic protein (GFAP), heat shock protein – 70 (Hsp-70) expression and apoptosis by transferase-mediated dUTP nick-end labeling (TUNEL) analysis.
**Results.** The number of cells with NF (26,0 ± 3,0 vs 16,1 ± 3,3; p<0,05), GFAP (12,0 ± 1,3 vs 3,2 ± 0,9; p<0,05) and Hsp-70 (10,0 ± 1,6 vs 4,2 ± 1,0; p<0,05) expression, were larger in the PRF side comparing with the control side. Additionally, glial cells in spinal ganglia of both sides demonstrated immunoreactivity. The instances of apoptosis were not significantly different, in statistical terms, between the control and experimental sides (18,0 ± 4,0 vs 20,0 ± 4,0; p=0,35).
**Conclusions.** PRF in spinal gangliocytes of lumbar region increases neural tissue cytoskeleton factors like NF and GFAP suggesting about active regeneration processes into the cells 1 month after the procedure.
Spinal gangliocytes one month after PRF treatment notably increases Hsp-70 expression suggesting about activation of cellular activity and inhibitory role reducing of oxidative stress.
Similar number of apoptotic cells in spinal ganglia of lumbar region after PRF and control side suggests about inhibitory role of PRF on programmed cell death and stimulation of cell survival.
**Key words:** pig, morphology, pulsed radiofrequency, dorsal ganglion root, growth factors-apoptosis, stress markers

## INTRODUCTION

Radiofrequency (RF) is a percutaneous minimal invasive procedure, for chronic pain management, which can be used, when conservative treatment modalities, including medical and physical therapy are not effective. Nowadays, in clinical practice, pain physicians applied two modes of radiofrequency procedures: continuous or conventional RF (CRF) and pulsed RF (PRF). The application of PRF in management of chronic pain is a useful tool, because it's low invasive character, the target selective approach, the possibility of outpatient treatment and its safety.

The first use of RF in humans, treating trigeminal neuralgia, dates back from 1931, when direct current of 350 mAmp was delivered through a needle with 10 mm uninsulated tip[16]. This technique produced nerve tissue lesion with unpredictable size, which can be dangerous and lead to complications. In 1965 Rosomoff et al.[23] were described percutaneous lateral cordotomy for unilateral malignant pain. Shealy reported the first use of RF current for spinal pain[25]. He was performed RF lesioning of the medial branch of the dorsal ramus for facets joint pain, using 14G electrode introduced through a 12G needle. It may produce mechanical lesions besides the desired thermolesions, due to large needle diameter. In 1977 Uematsu described the RF lesion of the dorsal root ganglion (DRG), using the same electrode as Shealy and 75°C tip temperature, causing serious damage of the DRG[37]. A turning point came in 1980 when small-diameter electrodes for the treatment of spinal pain were introduced[28]. The system, known as Sluijter Mehta Kit (SMK) system, consists of 22G disposable cannula with a fine thermocouple probe inside. The small electrode size and temperature control, allows performed procedures with less risk for nerves mechanical injury. After that, the main idea was to create a method to apply RF at

sufficient intensity without letting the tip temperature rise to neurodestructive levels. This method has been named PRF. The history of pulsed radiofrequency usage in chronic pain management originated in 1998, when Sluijter et al. applied it to the dorsal root ganglion[27]. In this modified technique in 1 second 2 bursts of 20 ms each RF current are usually delivered. One cycle has active phase (20 ms) and silent period (480 ms) to allow for washout of the generated heat. During the procedure the temperature of tissues around not exceed 42°C, however output is usually set at 45V and as result not causing damage to nervous tissue.

Analgesic mode of action of CRF consist of destroying nerves and subsequently disrupt the transmission of pain signals through the spinothalamic tracts of the spinal cord. The effectiveness of PRF was demonstrated in various good quality randomized control studies, but mechanisms of action are still unclear.

In an animal study evaluating the histologic effects of pulsed and continuous RF at 42°C to rat DRG and sciatic nerve, showed no structural changes, but cause transient endoneurial edema and collagen deposition. Tissue returned to normal conditions by 7 days in nerve and 21 days in the DRG[21]. In another study PRF applied to dorsal root ganglia induces cellular stress as measured by expression of neuron activating transcription factor 3, sensory fibers appear to be selectively targeted by it[8]. PRF has been introduced as non-neurodestructive procedure, however should partly destroy the myelin envelope of nervous fibers[22]. It was demonstrated using transmission electron microscopy of rats DRG at acute stage after PRF. In the similar study on ultrastructural changes showed microscopic damage after PRF exposure including abnormal membranes and morphology of mitochondria and disruption, which says disorganization of microfilaments and microtubules[6]. Nowadays is popular theory that rapidly changing electric fields produced by PRF alter the transmission of pain signals via a pathway involving c-Fos, a so-called immediate early gene, in the superficial laminae I and II of the dorsal horn at the C5 and C6 levels[38]. Authors were observed a significant increase of c-Fos expression in the dorsal horn of rats that underwent active intervention compared with the sham-operated controls.

There are two types of cell death: necrosis and apoptosis. They are generally considered to be distinct forms of cell death. Both were initially identified based on characteristic changes in cell morphology[14]. Necrosis can result from oxidative stress, but apoptosis can be induced by activation of cystein proteases, cell surface receptor engagement, growth factor withdrawal, and DNA damage. Apoptosis or programmed cell death (PCD) is genetically determined process to destroy cells for the maintaining of cellular homeostasis in the tissue. Gangliocytes death is normal during nervous system development but is abnormal in disease or injury. It can lead to the nerve tissue degeneration. Apoptotic nerve cell death now appears as likely to underlie a number of neurological conditions including Alzheimer's disease, Parkinson's disease, stroke, hereditary retinal dystrophies and Amyotrophic Lateral Sclerosis. Despite subsequent development of numerous molecular markers, the morphological changes still remain the "gold standard" to define the mode of cell death[4]. In light microscopy dorsal root ganglion neurons can be classified into A- and B-cells based on their distinct morphological manifestations. A-cells are big and normally have one big central-located nucleolus, whereas B-cells are small and have more than one peripheral-located nucleoli[32]. A- and B-cells have different physiological functions. A-cells project myelinated fibers that mediate proprioceptive sensations, whereas B-cells give thin-myelinated or unmyelinated fibers, which transmit nociceptive sensations[17,33]. We are particularly interested in the role of apoptosis after DRG PRF. To evaluate the level of apoptosis transferase-mediated dUTP nick-end labeling (TUNEL) analysis was performed. The method of TUNEL was tested on a variety of tissues in which the migration of cells to their final destination is already delineated unequivocally or in tissues that are known for their active PCD. The results demonstrate that in many of these examples, where traditional criteria of apoptosis are missing, the topographical arrangement of nuclei labeled for DNA breaks by TUNEL is in perfect agreement with the expected location of PCD[40].

Glial fibrillary acidic protein (GFAP) is expressed in the central nervous system (CNS) and it has proved to be the most specific marker for cells of astrocytic origin. GFAP is the principal 8-9 nm intermediate filaments in mature astrocytes. As a member of the cytoskeletal protein family, GFAP is thought to be important in modulating astrocyte motility and shape by providing structural stability to extensions of astrocytic processes. Although the exact role of GFAP is unknown, it is involved in many important CNS processes and is partially responsible for neurological functions within the blood-brain barrier. GFAP has been shown to play a role in mitosis by adjusting the filament network present in the cell. During mitosis, there is an increase in the amount of phosphorylated GFAP, and a movement of this modified protein to the cleavage furrow[34]. Studies have also shown that GFAP knockout mice undergo multiple degenerative processes including abnormal myelination, white matter structure deterioration, and functional/structural impairment of the blood-brain barrier[19]. Biochemical studies of GFAP have shown MgCl2 calcium/calmodulin dependent phosphorylation at various serine or threonine residues by protein kinase C and protein kinase A[9], which are two kinases that are important for the cytoplasmic transduction of signals. These data highlight the importance of GFAP for cell-cell communication. Following injury to the human CNS caused by trauma, genetic disorders, or chemicals, astrocytes proliferate and show extensive hypertrophy of the cell body and processes, and GFAP is markedly upregulated. In contrast, with increasing astrocyte malignancy, there is a progressive loss of GFAP production[5].

There are multiple disorders associated with improper GFAP regulation, and injury can cause glial cells to react in detrimental ways. Glial scarring is a consequence of several neurodegenerative conditions, as well as injury that severs neural material. The scar is formed by astrocytes interacting with fibrous tissue to re-establish the glial margins around the central injury core[2] and is partially caused by up-regulation of GFAP[30].

Another condition directly related to GFAP is Alexander disease, a rare genetic disorder. The cellular mechanism of the disease is the presence of cytoplasmic accumulations containing GFAP and heat shock proteins, known as Rosenthal fibers[7]. Notably, the expressions of some GFAP isoforms have been reported to decrease in response to acute infection or neurodegeneration[12]. Reduction in GFAP expression has also been reported in Wernicke's encephalopathy[3]. Changes in GFAP expression have been reported in Down's syndrome, schizophrenia, bipolar disorder, depression[12], Hiv-1[18], varicella zoster[13].

Neurofilaments (NF) are the intermediate filaments of nerve cells and one of the major components of the neuronal cytoskeleton. The function of these cytoskeletal elements is the control of axonal caliber. Neurons with large diameter axons contain more NF, comparing with small diameter axons. Particularly motor neurons contain big amount of NF, where fast impulse conduction play important role. They can accumulate as a marker of a disease process. NF accumulations are seen in several neurological diseases, including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and diabetic neuropathy[26,1,11,24].

Heat shock protein – 70 (Hsp-70) one of the heat shock proteins, which play vital role in protein folding and turnover. HSPs are traditionally classified into two groups: the high-molecular-weight HSPs and the small HSP family. To the first group belong the HSP90, HSP70, and HSP60 families. Under stress inducing conditions, production of many heat shock proteins increase dramatically in an attempt to protect cells from the effects of the stress inducing agent. They are preferentially induced in response to cell stresses including heat shock, oxidative stress, ultraviolet radiation, ischemia-reperfusion injury, viral infections, nutrient deprivation, and chemicals protecting cells from injury[20]. Hsp70 was demonstrated to have a disease suppressive role in experimental models of autoimmunity preventing disease[39] development and inflammatory effects, by the induction of IL-10 producing T cells.

## AIM OF THE STUDY

According that dorsal root ganglia is the first structure of pain modulation, the aim of our study is to analyse the histological effects of PRF on the domestic porcine dorsal root ganglion (DRG), and evaluate the expression of biomarkers glial fibrillary acidic protein (GFAP), neurofilaments (NF), heat shock protein 70 (hsp-70) and apoptosis by TUNEL method in gangliocytes of the subject(s).

## MATERIALS AND METHODS

**General Conditions**. A protocol used in this study was approved by the Animal Care Ethics committee, Riga, Latvia (N 41, 2012.26.01). A total 3 clinical healthy adult female domestic porcine weighting 59 to 65 kg were used for this pilot study. Animals were housed in standard farm in Jelgava district, with access to food and water *ad libitum*, and brought for the experiment to the Faculty of Veterinary Medicine, Latvian University of Agriculture, Jelgava, Latvia.

**Pulsed Radiofrequency Procedure**. After premedication with Azaperon (4 mg/kg i/m), Atropine (0.02 mg/kg i/m), and Ketamine (10 mg/kg i/m), induction with Pentobarbital (6 mg/kg i/v) the subject's porcines were placed in prone position and were intubated. Before intubation animals has received Pentobarbital (6mg/kg i/v). The animal's backs were shaved and prepared with antiseptic (96% ethanol). Following inhalation of Isoflurane (2Vol%) under X-ray control (C-arm, Philips) RF needle (Radiofrequency Cannula 22 guage, 5 mm active tip, S-1005, NeuroTherm) was introduced transforaminaly in order to maximally reach the DRG. Motor stimulation (2Hz) was positive between 0.5V-0.8V. The porcines were observed for muscles contraction. DRGs PRF was performed using a 10 cm long electrode and following RF generator (NeuroTherm 1100) settings: 42°C, 7 min, 5 p.p.s, 5ms, 45V. The temperature during all procedure was not exceeding 42°C and impedance was checked (180-220Ω). Four lumbar DRGs ($L_1$, $L_2$, $L_3$, $L_4$) were randomly treated. The opposite side DRGs was used as control.

**Observation of the Animals and Tissue Preparation**. Animals were checked daily for general condition and complications. The housing room was with 12h:12h light: dark cycle (lights on at 07:00 AM). 30 days after the procedure the animals was euthanized using pentobarbital (200 mg/kg, i/v). The lumbar region of the spine was separated and placed in a 10% buffer of formaldehyde for a month. After these fixations, using both sides' Th12-L5 hemilaminectomy methods, corresponding DRGs were prepared for slide analysis. They were embedded in paraffin in order to obtain 3**m**m thick sections, which were then cut by microtome and collected on slide glasses.

**Immunohistochemistry**. Multiple 3μm-thick sections of the paraffin-embedded DRGs were examined for immunohistochemistry. Prior to immunostaining, sections were deparaffinised and rehydrated. Sections were processed in microwave for 20 min in 4% citrate buffer (pH 10), quenched for 10 min with 3% $H_2O_2$ to block endogenous peroxidase activity, rinsed in phosphate-buffered saline (pH 7.4), pre-treated with nonimmune goat serum for 10 min to block a nonspecific antibody binding and than incubated for 2 h with the primary antibodies. The primary antibodies utilized in immunohistochemistry were monoclonal mouse anti-human neurofilament protein (NF, code-Nr. M 0762, dilution 1:100, DakoCytomation, DK), monoclonal mouse anti-human glial fibrillary acidic protein (GFAP,

code-Nr. M 0761, dilution 1:100, DakoCytomation, DK), mouse anti-HSP70 (HSP70, code-Nr. 33-3800, dilution 1:50, Invitrogen, UK).

TUNEL reaction was used for detection of apoptosis. In situ Cell Death Detection, POD (TUNEL, code-Nr. 11684817910, dilution 1:10, Roche, DE) was used. Deparaffinised sections (xylol 2 x 4 min, 99% ethanol 2 x 2 min, 95% ethanol 2 x 2 min and 70% ethanol 2 x 2 min) were rinsed with water (7-10 min) and transferred to PBS (pH 7.5) for 10 min. Subsequently slides placed into 50 ml PBS solution with 500 µl 30% hydrogen peroxide for 30 min on shaker to block the endogenous peroxidases. Afterwards tissue samples were washed with PBS (3 x 5 min), placed into microwave (700 W) for 10 min for fixation of antigen cooled to room temperature and rinsed with PBS. After that, slides were kept in refrigerator in 0.1% BSA (bovine serum albumin) solution with PBS for 10 min and than incubated in TUNEL mix (Tdt – mix of terminal deoxynucleotide transferase and DIG-labeled deoxynucleotide) for 1h at 37°C. Then the slides were rinsed with PBS 1:10, and incubated for 30 min at 37°C with POD (sheep anti-digoxygenin antibody coupled with horseradish peroxidase Fab fragment). Then the slides were washed with PBS, covered with DAB (diaminobenzidine chromogen) for 7 min, and then rinsed with running water for 5 min. Finally, haematoxylin and eosin staining was performed on each sample. Sections were covered with a polystyrene-based medium and coverslipped.

**Analysis**. For quantitative analysis we used a counting of cells with biomarkers expression, using light microscopy, in three fields of vision with X400 magnification. Counting was done by a blinded and experienced investigator. For all analysis significance level of <0,05 was applied. Obtained averages were compared for control and experimental group using t-test (either for equal variance for NF, or unequal one for GFAP and Hsp-70). For parameter TUNEL, the p-value was evaluated as probability to get total number of apoptotic cells in a whole series of probes.

## RESULTS

DRG PRF increase expression of biomarkers NF, GFAP, Hsp-70. The number of apoptotic cells does not change with treatment.

The number of cells with NF, GFAP and Hsp-70 expression, were larger in the PRF side as compared with control side ($p< 0,05$) (Table 1).

Generally number of NF–containing gangliocytes was higher in the experimental side (26,0 ± 3,0) then in the control side (16,1 ± 3,3) (Figs. 1-2).

The same relation we observed also for GFAP positive gangliocytes in experimental side (12,0 ± 1,3) and in the control side (3,2 ± 0,9) (Figs. 3-4). Additionally, glial cells in spinal ganglia of both sides also demonstrated immunoreactivity. Cellular stress marker Hsp-70 showed obvious dominance of factor positive cells in the experimental side (10,0 ± 1,6) (Fig. 5) in comparison to the control side (4,2 ± 1,0) (Fig. 6).

Apoptosis appeared in approximately similar numbers in both control (18,0 ± 4,0) and experimental (20,0 ± 4,0) sides (Figs. 7-8) (Table 1) without any statistical difference between ganglias (p=0,35).

## DISCUSSION

PRF is widely used for the treatment of chronic pain, although its mechanism of action is still not known. However, PRF was advocated as a non-destructive pain therapy, on the basis of the fact that patients treated with pulsed radiofrequency did not show clinical signs of nervous tissue destruction[29]. In our experiment we have not found changes in apoptosis after DRG PRF in the animals that were sacrificed 30 day after procedure. Thus, we suggest about PRF inhibitory effect on spinal ganglion that avoid cell death and stimulate cells survival. This is the first report about apoptosis research on PRF affected neuronal tissue and results seem to be very original and promising of view of tissue surviving. Recently, Podhajsky RJ et al[21] reported that exposure of the DRG in rats to PRF currents causes only transient endoneurial edema, without others structural changes, which resolved on day 21 after procedure. Higuchi et al[10] reported that exposure of the DRG in rats to PRF currents activates dorsal horn lamina I and II neurons, and this effect is not mediated with tissue heating. However, Erdine at al[6] demonstrated abnormal disruption of mitochondria, especially in smaller pain-carrying C-fibers.

Our study has shown multiple NF-containing gangliocytes in experimental side and small amount of NF-containing positive gangliocytes in control side. As NF is an important component of the neuronal cytoskeleton, our data suggest that NF accumulation after PRF might underlie disruption of axonal transport, with probably following worsening of pain signals transmission.

We found GFAP expression in gangliocytes, which was higher in treated side, comparing with untreated. It is the first biomarker, which react on nerve cells stress. Loss of GFAP impairs neuronal cells proliferation and delays nerve regeneration after damage[35]. According to this fact, we suggest about PRF regeneration affinity on gangliocytes.

We also found Hsp-70 expression in gangliocytes, which was significantly higher in PRF treated side, comparing with untreated. Hsp-70 was demonstrated to have a disease suppressive role by reducing of oxidative stress and inflammation. In experimental models in other authors was demonstrated reduction of inflammatory responses against Listeria monocytogenes via production of IL-10[15]. Via the same mechanism, another study confirmed that Hsp-70 protected rats from development of arthritis[34]. This finding indicates that the effect of PRF on the nerve tissues is anti-inflammatory and could explain PRF effectiveness in such pathologies like radicular pain and peripheral neuralgias, where inflammation of DRG or nerve root can be caused by injury or influence of inflammatory cytokines and others biologically active substances. In

addition, Hsp-70 has been reported to play important role activation in lymphocytes and macrophages, and provide the link between innate and adaptive immune system[36]. In another study suppression of metastatic tumor progression after immunization of mice with Hsp (gp96) has been introduced[31]. Due to this fact, it is not excluded that PRF activate also tissue immune response.

## CONCLUSIONS

1. PRF in spinal gangliocytes of lumbar region increases neural tissue cytoskeleton factors like NF and GFAP suggesting about active regeneration processes into the cells one month after the procedure.
2. Spinal gangliocytes one month after PRF treatment notably increases Hsp-70 expression suggesting about activation of cellular activity and inhibitory role reducing of oxidative stress.
3. Similar number of apoptotic cells in spinal ganglia of lumbar region after PRF and control side suggests about inhibitory role of PRF on programmed cell death and stimulation of cell survival.

**Conflict of interest:** None

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**Table 1. Mean number and standard deviation of different factors and apoptosis positive cells in the control and the experimental pigs spinal ganglia one month after PRF.**

| Factors | Control | Experimental | p-value |
|---|---|---|---|
| NF | 16,1 ± 3,3 | 26,0 ± 3,0 | <0,05 |
| GFAP | 3,2 ± 0,9 | 12,0 ± 1,3 | <0,05 |
| Hsp-70 | 4,2 ± 1,0 | 10,0 ± 1,6 | <0,05 |
| TUNEL | 18,0 ± 4,0 | 20,0 ± 4,0 | 0,35 |

Abbreviations:
NF – neurofillament
GFAP – glial fibrillary acidic protein
Hsp-70 – heat shock protein 70
TUNEL – transferase-mediated dUTP nick-end labeling analysis

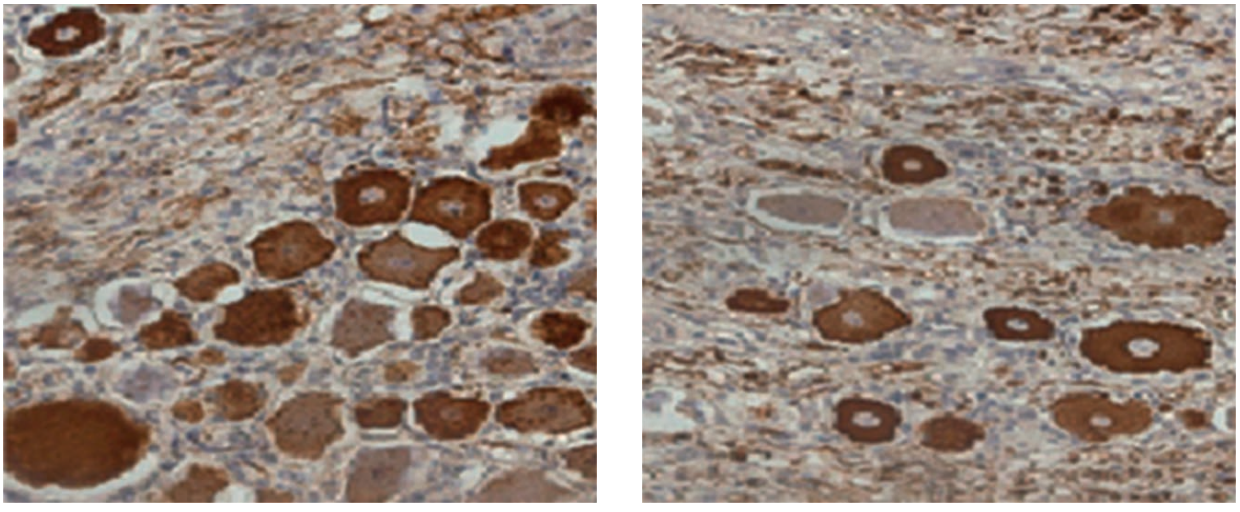


**Fig. 1-2: multiple NF containing gangliocytes (1, L2dxt., ×400, NF IMH) in experimental side and small amount of NF positive gangliocytes - control side (2, L2sin., ×400, NF IMH) one month after PRF.**

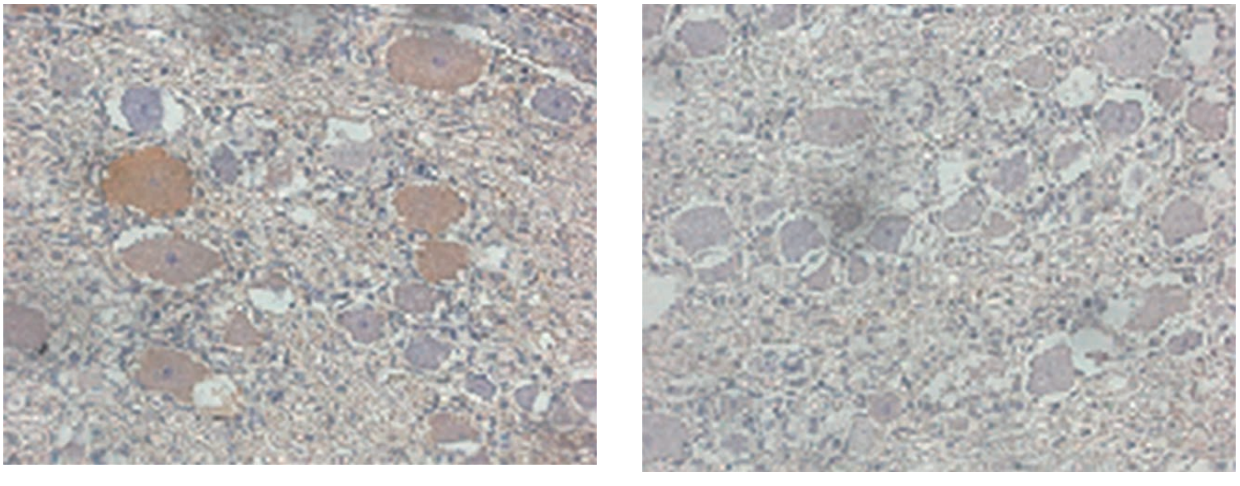
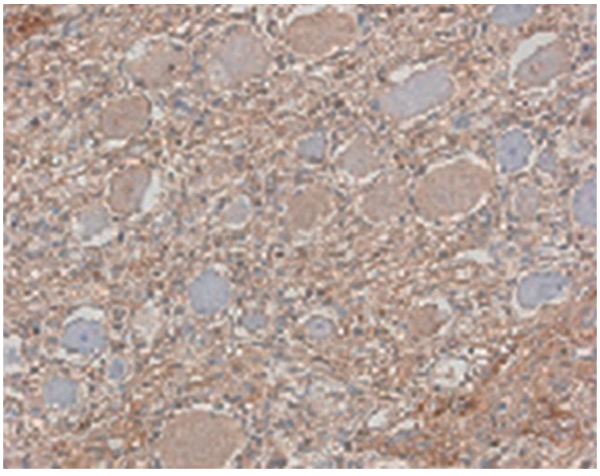


**Fig. 3-4: multiple GFAP containing gangliocytes (3, L4dxt., ×400, GFAP IMH) in experimental side and small amount of GFAP positive gangliocytes - control side (4, L4sin., ×400, GFAP IMH) one month after PRF.**



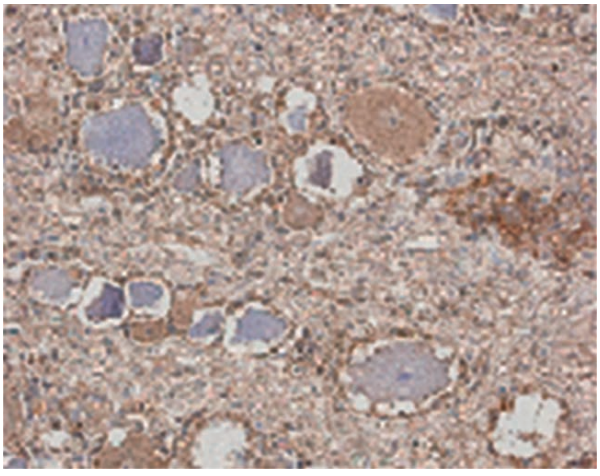
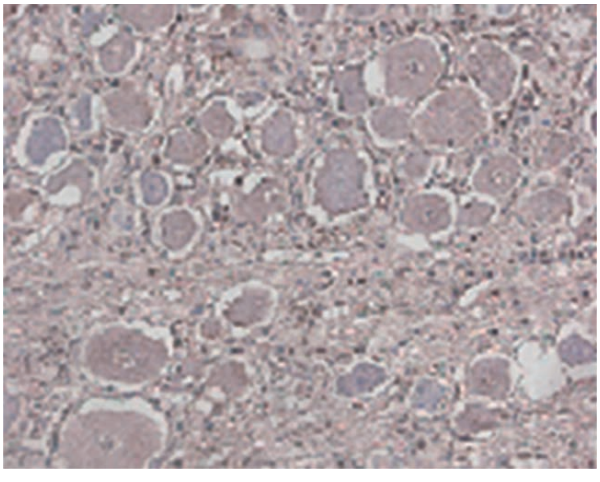


**Fig. 5-6: multiple Hsp-70 containing gangliocytes (5, L4dxt., ×400, Hsp-70 IMH) in experimental side and small amount of Hsp-70 positive gangliocytes - control side (6, L4sin., ×400, Hsp-70 IMH) one month after PRF.**



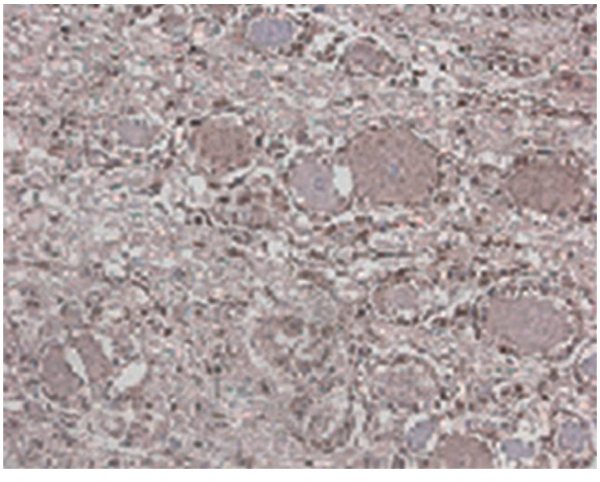


**Fig. 7-8: note absence of difference in appearance of apoptotic cells both – experimental (7, L3dxt., ×400, TUNEL) and control (8, L3sin., ×400, TUNEL) sides in spinal ganglia one month after PRF.**

10.2478/v10163-012-0012-x



*PROBLEM-SOLVING ARTICLE*

# Pathology of Breast Cancer: from Classic Concepts to Molecular Pathology and Pathogenesis

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**Summary**
Breast cancer has high incidence and still significant mortality. Due to the widespread application and efficacy of surgery in breast cancer treatment, the surgeon has a crucial role in the treatment planning. Taking into account the tendency to personalized cancer care and the heterogeneity of breast cancer, the surgeon has to be aware about the prognostic and predictive characteristics of breast cancer. We discuss here the classaic pathology of breast cancer along with molecular subtypes, novel prognostic markers and molecular pathogenesis.
**Key words:** breast cancer, pathology, molecular pathology, molecular subtypes, immunohistochemistry

## INTRODUCTION

Tissue examination is the gold standard in the tumour diagnostics. Depending on the submitted tissue material, pathology can reveal the presence and spread of the tumour as well as characterise the biological potential as benign or malignant. Up-to-dated techniques and integrated approach to tissue evaluation along with other scientific methods can bring higher volumes of information with high clinical relevance. The morphologic data can predict the potential effect of different treatment modalities. The pathogenesis of tumour also is partially reflected in the neoplastic tissues. a

Breast cancer represents one of the best studied malignant tumours. Considering breast cancer, awareness of pathology is practically important in surgeon's work for planning the treatment. Breast cancer research provides also bright evidence of the possibilities of tissue and integrated investigations in oncology. The level of knowledge in this field could facilitate the development of medical science regarding other malignancies.

The aim of the article is to highlight the classic and modern concepts of breast cancer pathology having clinical implications and / or prognostic value.

## DISCUSSION

### Breast cancer in surgical practice

Breast cancer is one of the most common malignant tumours in the European population and the most frequent malignancy in female (Bombonati and Sgroi, 2011). Surgery has an important role in the treatment of the primary tumour. In selected cases, patients with metastatic disease also can benefit from surgical treatment (Guarneri and Conte, 2009). However, as the treatment of breast cancer is complex, including surgery as a crucial but not the only step, wider understanding of breast cancer biology is necessary.

### Classic pathology of breast cancer

The classics of breast cancer characteristics are represented in the classification of breast tumours by the World Health Organization (Malhotra *et al*., 2010). Traditionally, breast cancer is characterised as *in situ* or invasive regarding the integrity of basement membrane in the former case or loss of it in the second case. This concept is major prognostic value (Bombonati and Sgroi, 2011). At present, cancer *in situ* is described as ductal or lobular. The invasive cancers (listed in Table 1) are classified into ductal, lobular, medullary and other, less frequent types (Figure 1). This classic classification retains prognostic importance and must be invariably applied when evaluating malignant breast tissue.

Besides that, several specific morphological breast cancer types can be associated with specific problems in diagnostics and treatment. Lack of cell cohesion in case of lobular cancer can lead to widespread, still clinically and radiologically silent spread of tumour (Figure 2). Both medullary and mucinous cancer can negatively interfere with diagnostics due to softer consistency by palpation as well as clinical and radiological circumscription in case of medullary cancer, and lower sensitivity of fine needle aspiration (FNA). FNA diagnostics is embarrassed by significant inflammatory infiltrate in medullary cancer as well as by low cellularity and usually low grade in mucinous cancer.

**Table 1. Histologic types of invasive breast cancer: characteristics and clinical significance**

| Histologic type | Frequency, % | Characteristic features | Clinical importance |
|---|---|---|---|
| Ductal cancer | 40 – 75 | Tubule formation, cellular atypia and mitotic activity are grade-dependant<br>Necrosis can be present<br>Amount of stroma is variable | The most frequent type of breast cancer |
| Lobular cancer | 3.2 – 14<br>Greatly depends of the applied pathologic criteria | Lack of cellular cohesion<br>• Frequent truncation mutations in *E-Cadherin* gene<br>• Lack of E-Cadherin protein expression by immunohistochemistry<br>• Individual growth of tumour cells or arrangement in files | "Skip lesions" result in higher risk of positive resection margins or unidentified incomplete resection; false impression of multifocality |
| | | Occasional lack of stroma | Difficulties in mammographic detection and / or palpation |
| | | Smaller cells<br>Low mitotic activity<br>Rare necrosis<br>Frequent intracellular mucin<br>More frequently ER+, PR+<br>Rarely HER2-positive or p53+ | More beneficial prognosis if compared with stage-matched ductal carcinomas |
| Tubular carcinoma | 2 – 5 | High differentiation:<br>• Tubular architecture (at least 90%)<br>• Lack of myoepithelial cells<br>• Little pleomorphism<br>• Low mitotic rate<br>• More frequently ER+, PR+. Rarely HER2-positive or p53+ | Favourable prognosis using strict criteria<br>Difficult morphologic differential diagnosis regarding radial scar and sclerosing or microglandular adenosis |
| Cribriform carcinoma | 2 – 4 | • Cribriform architecture<br>• Lack of myoepithelial cells<br>High differentiation:<br>• Little pleomorphism<br>• Low mitotic rate<br>• More frequently ER+, PR+. Rarely HER2-positive or p53+ | Favourable prognosis if adhering to strict criteria |
| Mucinous carcinoma | 2 – 3.6 | • Neoplastic cells surrounded by pools of extracellular mucus (100%)<br>• Lack of myoepithelial cells<br>High differentiation:<br>• Little pleomorphism<br>• Low mitotic rate<br>• Usually ER+, PR+, HER2-negative and p53-negative | Favourable or excellent prognosis if adhering to strict criteria |
| Medullary carcinoma | 1 – 5 | Syncytial growth (at least 75%)<br>Demarcated outline despite true invasive growth<br>Marked stromal infiltration of lymphocytes and plasmocytes<br>Frequent necrosis<br>Mostly ER-, PR-, HER2-negative: triple-negative molecular type<br>p53-positive | Better prognosis if adhering to strict criteria<br>Association with *BRCA1* mutation: histology can be key for genetic evaluation |

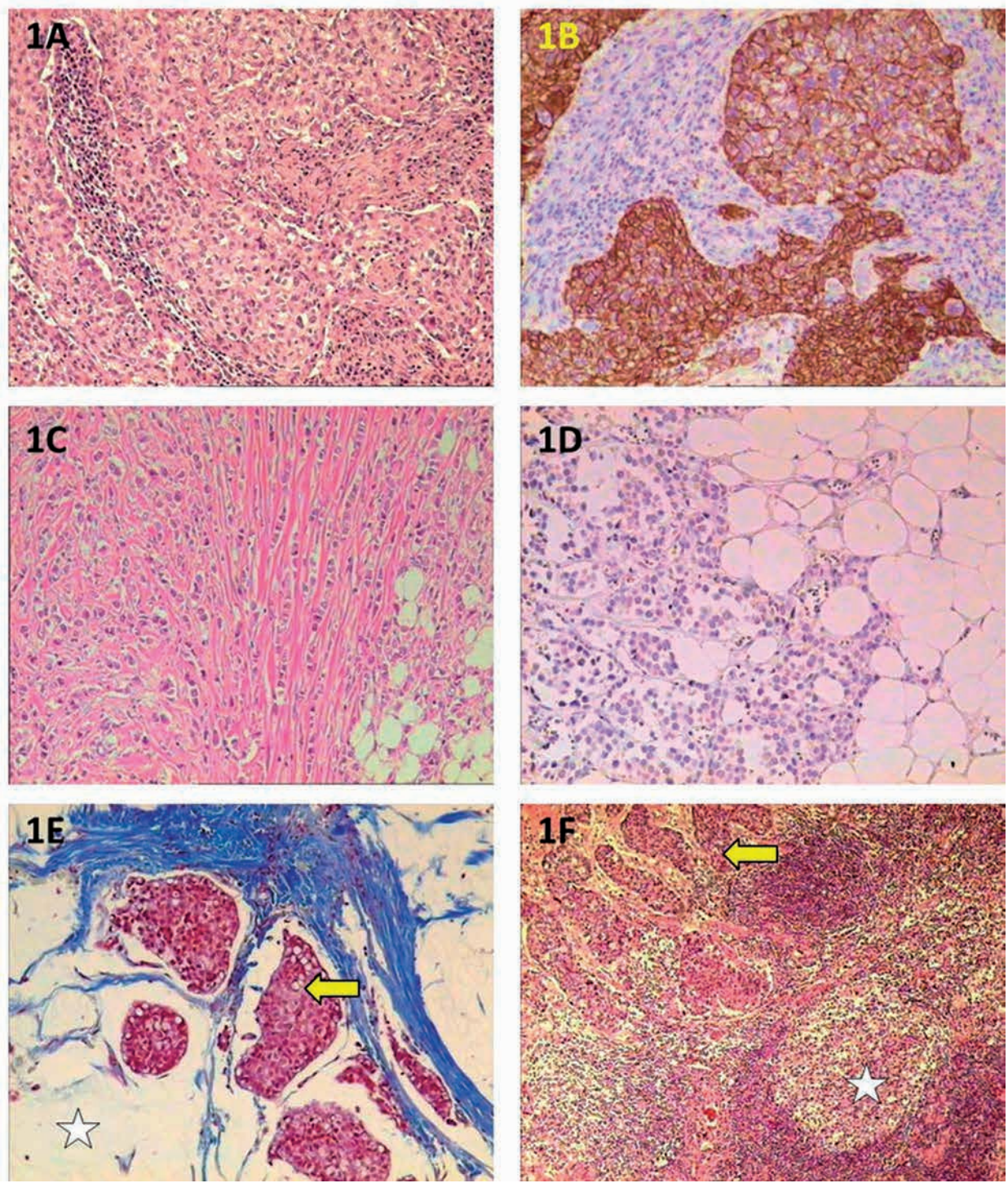


**Fig.1. Histological types of breast cancer. A, High-grade ductal cancer. Haematoxylin-eosin (HE), original magnification (OM) 100x. B, Membranous expression of E-Cadherin in ductal cancer confirming the histogenesis even in high-grade case. Immunoperoxidase (IP), anti-E-Cadherin, OM 100x. C, Lobular cancer. HE, OM 100x. D, Lack of E-Cadherin in lobular cancer. IP, anti-E-Cadherin, OM 100x. E. Mucinous cancer. Note the abundance of mucus (star) and lower amount of neoplastic cells (arrow). Masson's trichrome, OM 100x. F, Medullary cancer. Note the presence of lymphoid follicle (star) as well as neoplastic growth (arrow). HE, OM 50x.**

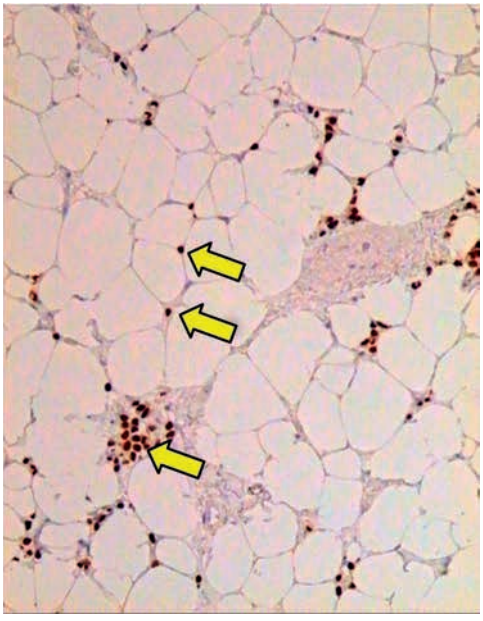

**Fig. 2. Marked invasive growth of lobular breast cancer. The tumour cells are highlighted by arrows. IP, anti-estrogen receptor alpha, OM 100x.**

**Molecular pathology of breast cancer**

Breast cancer is a heterogeneous disease including several entities with different clinical behaviour. Even tumours belonging to the same histologic type can have different clinical course. Naturally, the largest group – ductal cancer – shows the highest heterogeneity. Additional information can be obtained from molecular subtyping of breast cancer. This approach is based on expression patterns of so called intrinsic genes (Perou *et al.*, 2000) and results in breast cancer classification into subgroups with different biological properties and response to treatment. The intrinsic genes were defined as genes with higher variation of expression between tumours than within one tumour (Strehl *et al.*, 2011). The genes in breast cancer became up-regulated or down-regulated in larger groups, as will be described further for each molecular subtype. At present, molecular subtyping of breast cancer has become routine practice. The molecular subtypes initially were discovered by gene expression profiling in high throughput microarray technologies (Perou *et al.*, 2000). At present, immunohistochemistry (IHC) is accepted as adequate surrogate marker (Nielsen *et al.*, 2004; Carey *et al.*, 2006) benefitting from higher economic effect and simpler technology despite less robust data in predictive sense (Sorlie, 2004)

The best-known molecular subtypes of breast cancer include luminal or hormone-sensitive, HER2-positive and triple negative tumours (Guarneri and Conte, 2009). The division of luminal subtype into luminal A and luminal B is also well-accepted. The basal or basal-like breast cancer is a matter of active discussions as it overlaps with triple-negative subtype but is not synonymous with it. The other described molecular subtypes include normal-breast like and molecular apocrine subtype.

The luminal molecular subtype (Figure 3) is characterised by estrogen (ER) and progesterone (PR) receptor positivity (Strehl *et al.*, 2011). Luminal subtype can be classified into luminal A and B subtype. The prognostically worse luminal B subtype can be recognised by co-expression of HER2 in addition to ER and PR in contrast to HER2-negative luminal A subtype, or by higher proliferative activity (Cheang *et al.*, 2009; Nielsen *et al.*, 2010; Strehl *et al.*, 2011). In our opinion, diagnostics of luminal B subtype by higher proliferative fraction (reaching or exceeding 14%, as described by Goldhirsch *et al.*, 2011), is less subjective and thus more reliable.

HER2 positive breast cancer (Figure 3) lacks expression of ER and PR, but is defined by HER2 protein over-expression by immunohistochemistry and/or *HER2/neu* gene amplification by *in situ* hybridisation (Strehl *et al.*, 2011). Breast cancer negative for ER, PR and HER2 protein expression is called triple negative (Figure 3). It partially overlaps with basal-like subtype showing expression of basal cytokeratins that normally are present in the basal cell of mammary ducts. High proliferative activity is typical.

New molecular subtypes have also been described. The claudin-low subtype includes triple negative breast cancers lacking also ytokeratin 5/6 and epidermal growth factor receptor in contrast to basal triple negative subtype (Prat *et al.*, 2010; Strehl *et al.*, 2011). The molecular apocrine breast cancers are characterised by ER negativity and androgen receptor positivity in addition to apocrine morphology with presence of intracellular vacuoles (Farmer *et al.*, 2005). In contrast, the initially described normal-breast like subtype is suggested to be the result of specimen contamination by normal tissues (Parker *et al.*, 2009; Weigelt *et al.*, 2010; Strehl *et al.*, 2011).

Each molecular subtype has different biological properties and clinical course. Luminal breast cancer has generally better prognosis (Sorlie *et al.*, 2001; Strehl *et al.*, 2011). It responds to hormonal treatment but show lower chemosensitivity (Rouzier *et al.*, 2005; Peppercorn *et al.*, 2008; Parker *et al.*, 2009; Strehl *et al.*, 2011). Luminal cancer has tendency to relapse in bone or soft tissues. Both HER2-positive and triple negative breast cancer has higher tendency to early development of metastases in visceral location or central nervous system (Guarneri and Conte, 2009). The molecular type also serves as guide for treatment: luminal type can be targeted by hormone therapy, HER2-positive tumours – by anti-HER2 agents, and triple negative – by chemotherapy. Triple-negative breast cancer cells also are dependant of poly (ADP) ribose polymerase (PARP) to repair single strand breaks in DNA, therefore PARP inhibition can be effective treatment modality (Guarneri and Conte, 2009).

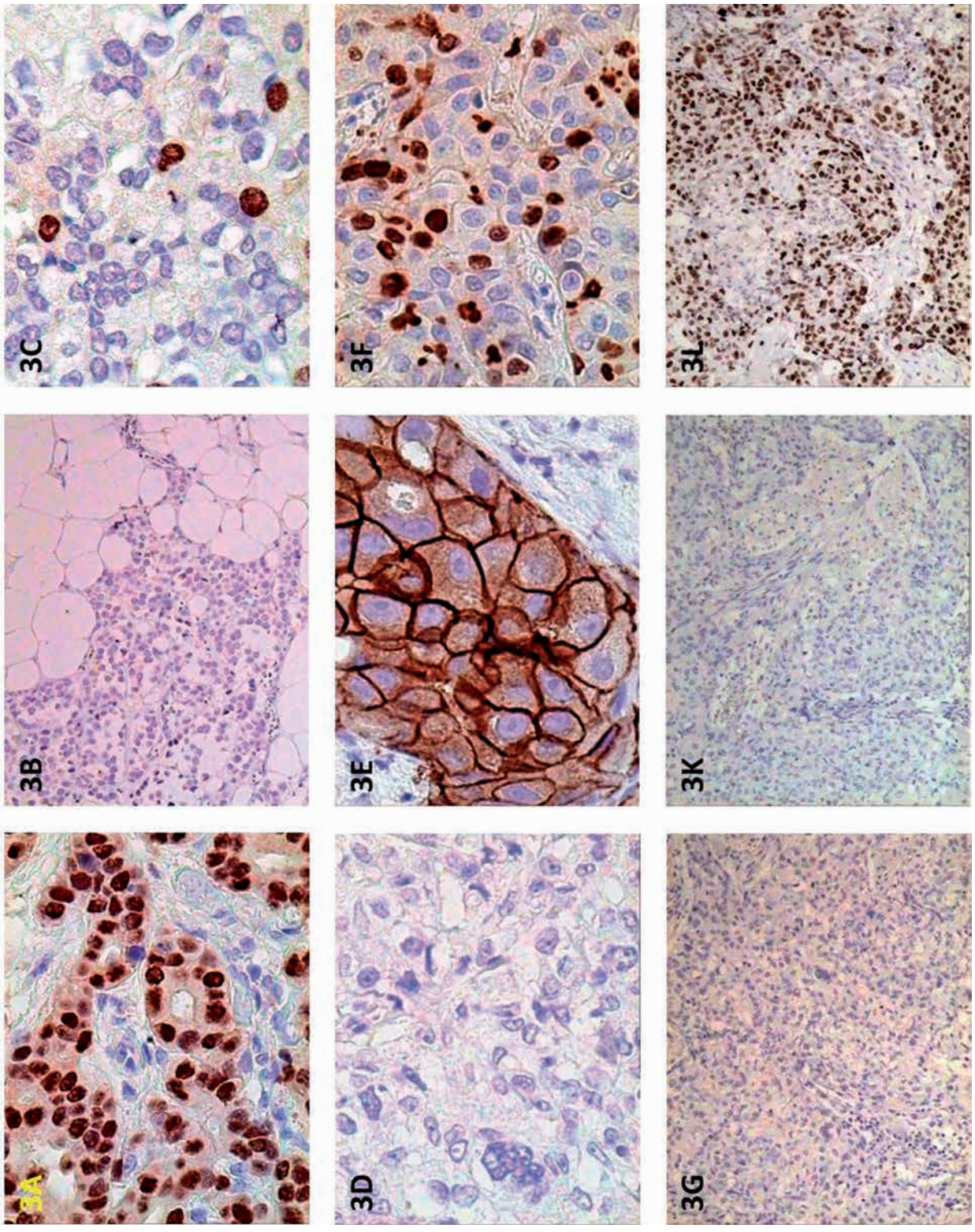


**Fig. 3. Molecular subtypes of breast cancer. A-C, Luminal breast cancer. A, Estrogen receptor expression. B, Lack of HER2 protein. C. Low proliferation fraction. D-F, HER2 overexpressing breast cancer: D, lack of estrogen receptors; B, HER2 protein overexpression; C, Moderate proliferative fraction. G-L, triple negative breast cancer: G, lack of hormone receptors; K, lack of HER2 protein; L, high proliferative fraction. Immunoperoxidase, A, D and G, anti-estrogen receptor alpha; B, E and K, HercepTest; C, F and L, anti-Ki-67. OM 100x (B, G-L) and 400x (A, C-F).**

The molecular subtype along with other factors as tumour size, lympho-vascular invasion, and age at diagnosis is found to influence the sentinel node positivity (Reyal *et al*., 2011). The molecular subtype thus interacts with metastatic process and is an evidence of up-to-date investigation of biological potential. It also correlates with the local tumour recurrence (Voduc *et al*., 2010), response to neoadjuvant systemic treatment (Rouzier *et al*., 2005), metastatic pattern (Gabos *et al*., 2006) and survival (Weigelt *et al*., 2010).In addition, the molecular subtypes are related to different risk factors and differ by geographic distribution (Phipps *et al*., 2008). Thus, molecular subtyping of breast cancer identifies biologically different neoplastic processes with different clinical course and reaction to treatment.

**Other molecular and biologic factors**

The hot topics in breast cancer research include the wide and growing field of epigenetic research (Huang *et al*., 2011), investigation of microenvironment and breast adipocytes (Place *et al*., 2011; Tan *et al*., 2011) and studies of additional immunohistochemical factors. The studies of microenvironment concern myoepithelial cells, cancer-associated fibroblasts, matrix remodelling and infiltrating leukocytes as well as microenvironment of metastases in order to characterize prognosis and find new targets for treatment (Place *et al*., 2011). Novel molecular factors that might play role in breast cancer development, reveal prognosis and potentially become target for treatment, include fascin (Al-Alwan *et al*., 2011), matrix metalloproteinase-1 (Bostrom *et al*., 2011), cyclooxygenase-2 (Kang *et al*., 2011), interleukins (Iliopoulos et al., 2011), p53 (Malhotra *et al*., 2010), p27 (Wander et al., 2011) and apoptosis-related factors including Bcl-2 (Zaha and Lazar, 2012).

**Molecular pathogenesis of breast cancer**

Invasive breast cancer is preceded by several stages of *in situ* atypia, progressing to *in situ* cancer. There are at least 2 hypotheses of breast cancer origin: the sporadic clonal evolution model and the cancer stem cell model (Bombonati and Sgroi, 2011). The sporadic clonal evolution model describes the cancer development as accumulation of genetic and epigenetic changes in epithelial cells resulting in proliferation advantage. The stem cell model emphasize that normal breast stem cells accumulate the alterations due to prolonged lifetime of stem cells. The final pathogenetic way could incorporate elements from both models with accumulation of genetic mutations and epigenetic events in stem cells. It is also possible that progenitors of stem cell are the true cancer source; in this case the type of cancer would be dependent on the differentiation of progenitor cell (Nowell, 1976; Reya *et al*., 2001).

From pathologist's point of view, progression of malignancy to higher grade occasionally is evident. However, the genetic studies point towards association of several chromosomal aberrations with the grade (Roylance *et al*., 1999; Buerger *et al*., 1999). Loss of chromosome 16 is frequent in low-grade ductal and in classic lobular cancer, but rare in high-grade cancers. Other aberrations are described as well. The high-grade cancers are commonly characterised by loss of 13q, gain of chromosomal region 11q13, amplification of 17q12. *In situ* and invasive cancers share the aberrations by grade (Bombonati and Sgroi, 2011). Thus, low-grade and high-grade cancers seem to be more separated entities. It is estimated that 9% of high-grade cancers still develop from low-grade cancers (Allred *et al*., 2008; Natrajan *et al*., 2009). The further growth and metastatic spread are largely influenced by the molecular type. The most of molecular changes in the epithelium occur before invasion, but in stroma and microenvironment – during the transition from preinvasive to invasive cancer (Bombonati and Sgroi, 2011).

In conclusion, breast cancer is a heterogeneous group of tumours. In order to plan the treatment, histologic type and molecular subtype should be detected. To plan personalised treatment, knowledge about other, novel prognostic and predictive factors can be necessary.

**Conflict of interest:** None

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**ACKNOWLEDGEMENT**

This work was supported by ESF project Nr. 2009/0230/1DP/1.1.1.2.0/09/APIA/VIAA/070.

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10.2478/v10163-012-0013-9



*PROBLEM-SOLVING ARTICLE*

# Diagnosis and Management of Posttraumatic Pancreatits in Children

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**Summary**
Traumas take a significant place in the etiology of children's acute pancreatitis. Diagnostics and treatment of posttraumatic pancreatitis (PTRP) are complex. Delayed diagnosis of pancreatitis is connected with heightened morbidity and mortality. There is a remarkable difference in the tactic of treatment between adults and children because of the anatomical physiological reasons. Special attention has to be paid to children under the age of two years. The article summarizes the literature review and the experts' conclusions from various European countries. Children who have PTRP should be concentrated in the specialized centres. It is necessary to have international cooperation of multicenters to improve the diagnostics and treatment of children PTRP.
**Key words:** children, pancreatitis, pancreatic trauma, posttraumatic pancreatitis, diagnostics, treatment, complications.
**Abbreviations:** PTRP – posttraumatic pancreatitis, US – ultrasonoscopy, CT – computer tomography, MR – magnetic resonance, ERCP – endoscopic retrograde cholangiopancreatography, SIRS - systemic inflammatory response syndrome, MODS –multiple organ dysfunction syndrome.

## INTRODUCTION

Acute pancreatitis is an acute inflammation of the pancreas with variable severity from mild abdominal pain, which may go undiagnosed, to fulminant necrotizing pancreatitis and death. It is likely that there is a complete interval resolution on morphology and function as opposed to the occurrence of irreversible changes in the pancreas in cases of chronic pancreatitis. Fibrosis and infiltration of the chronic inflammatory cells can lead to exocrine or endocrine failure or both (18). In case of PTRP, especially in severe injuries with anatomical disintegration, intransient consequences of these traumas cannot be excluded; they might appear in adulthood having diseases requiring a compensatory pancreatic load. Children pancreatitis is more common than it is generally believed and can be associated with severe morbidity and mortality. The causes of an acute pancreatitis include trauma, biliary tract stone disease, choledochal cyst, ductal developmental anomalies, drugs, metabolic derangements, and infections. The cause is not apparent for about 35% of children and is called "idiopathic". Severe pancreatitis was most commonly associated with systemic disease and trauma. Numerous reports have identified an increasing trend in the diagnosis of acute pancreatitis for children and the key differences are in disease symptoms and management between infants and older children (16; 17; 21). The reasons for the increase are not entirely clear and may be multifactoral. The significance of the increase of severe infectious and systemic diseases, which have an acute pancreatitis as a complication have been mentioned (13; 17). Some publications relate it to the recent tendency to concentrate the patients in centres doing the corresponding research (21) and seeing significant correlation between the increasing numbers of amylases/lipases tests and increasing of acute pancreatitis (16). Traumas are wide spread (15-40%) in the etiology of children's acute pancreatitis (7; 10; 12; 17; 21; 27; 28; 29). Up to 10% of children, who have a blunt abdominal cavity trauma, have pancreatic injuries, and the frequency of these cases tends to increase (15). Taking into consideration the very significant proportion of the trauma mechanism related with a bump into the bicycle's handlebar and the adoption of bicyclists' traditions of the "old Europe" in Latvia, considering our road infrastructure and drivers' intelligence, the future does not seem careless. The pancreas is located retroperitoneal to the spinal column, these 2/3 of the injury are localized in the body of the pancreas. The vulnerability of child's pancreas is common due to softer tissues and weaker anatomic protection by the anterior abdominal wall and other organs. In comparison with adults, children more frequently get an isolated pancreatic trauma, which probably indirectly indicates greater vulnerability of pancreatic tissues. Probably the fact that children in general lack primary pathology of the pancreas is crucial for better treatment results at the child's age.

Preparing this article the cognitions gained in the review of literature were discussed with the experts from Children Clinical University Hospital in Riga, Health Sciences' Paediatric Surgery Clinic of the University of Lithuania o in Kaunas, Paediatric Surgery Clinic of the University of Amiens, France, Paediatric Surgery Clinic of Helios Klinikum Berlin- Buch, Berlin, Germany, where during the last 12 years altogether there have been treated over 90 patients with a pancreas trauma, more than 85 % of them are recognized as posttraumatic pancreatitis. The authors of the paper would like to express a particular gratitude for the professors V.

Barauskas (Kaunas), J. P. Canarelli (Amiens) and K. Schaarschmidt (Berlin) for sharing of their experience. Detailed research protocols about the children who have pancreas traumas have been filled in Riga during the last 2 years and the cytokine tests in 24, 48 and 72 hours after the trauma have been made to find correlations in the laboratory indicators, which along with the data of other research works (8; 9; 14; 22; 31) let recognize severe pancreatitis timely and adjust appropriate treatment tactics. Cytokines themselves do not induce pancreatitis but rather mediate the progression of pancreatitis. Pancreatic production of pro-inflammatory cytokines modulates a local injury systemic inflammatory response, and a distant organ failure, which, along with pancreatic necrosis, determines the outcome from an acute pancreatitis. Onset of cytokine production follows immediately after the onset of pancreatitis and peaks in 36 – 48 hours; cytokine antagonist therapy may represent a potential therapeutic target and therefore is of an intense interest (32).

**Diagnosis.** PTRP occurs in association with blunt abdominal trauma, child abuse, or penetrating wounds or after surgery**.** Trauma impact is necessary for the origin of PTRP; however, in less severe cases the anamnesis of the trauma might lack or it is considered to be inessential. In order not to miss out the trauma, purposeful questions shall be used including all people who take care of the child. It has to be assumed that teenagers might keep the trauma in secret. It is essential to find out the mechanism of the trauma, localization of its impact, force and duration in detail. The time of getting the trauma is important in order to apply laboratory tests in dynamics and CT the most productively. It has to be kept in mind that acute pancreatitis might develop after every seemingly insignificant abdominal trauma. Taking into consideration complicated clinical diagnostics and possible imprecision of laboratory and X-ray diagnostics, any patient of a child age having a blunt abdominal trauma, especially in case of a possible pancreatic injury, has to be hospitalized into the intensive therapy unit during the first 24 hours, where a careful monitoring and examinations in dynamics has to be performed in order to identify a more precise diagnosis, a degree of gravity of the injury and a possible necessity for surgical treatment.

Cardinal changes in the field of acute pancreatitis' diagnostics and treatment took place after the Atlanta (USA) Consensus Conference in 1992. These Atlanta criteria require that patients meet at least 2 of the fallowing 3 parameters to qualify as having an acute pancreatitis: a typical abdominal pain, elevated amylase/lipase> 3 times the upper limit of normal, and /or confirmatory findings on cross-sectional abdominal imaging (4). After this conference the classification of an acute pancreatitis was supplemented by the term SIRS and MODS (dysfunction of more than two organs named as multi-organ failure). This conception changed the essence of treatment from treatment of local characteristics mainly in a surgical way to an early complex intensive therapy having two major aims: to decrease the severity of SIRS ensuring the functions of all organs' systems and the adequate perfusion of all tissues and to reduce maximally the possibility to get the infection of pancreas and peri-pancreatic tissues. The main conclusion of the new strategy – to a large extent the condition of the patient is not so severely threatened by necrosis, purulence or exudate as a body reaction to this pathological condition. However, the experience gained in the treatment of adults cannot be completely applied to children. Difference in age, developmental stages, and environmental exposures for children may influence the presentation of children who have an acute pancreatitis.

Acute pancreatitis in children usually presents with the sudden onset of mid-epigastric pain continuous and dull in nature (80-95%). Diffuse abdominal pain was reported into for 12-20% of patients. The typical radiation of the pain to the back observed in adults is missing in 60-90% of children (13, 20, 28). Pain is associated with anorexia, nausea and vomiting (40-80%) (25, 28, 29). In severe disease, the child is irritable and assumes a flexed position. For nonverbal children, irritability was a common presenting complaint and may be a surrogate for complaints of pain in this age group. The abdomen may be distended with guarding and rigidity present (29-37%) (3, 11). In very severe cases a child may present with shock, respiratory distress or multi -organ failure or unexplained jaundice or ascites. In the cases of necrotizing or hemorrhagic pancreatitis, hemorrhage may dissect from the pancreas along the tissue planes, presented as ecchymosis either in the flanks (Grey-Turner sign) or at the umbilicus (Cullen's sign). The ecchymoses typically take 1 to 2 days to develop and are observed among children very rarely in comparison with adults. When necrosis, abscess or pseudocyst develop, the presentation may be with a mass in the epigastrium. The most common mass was an abdominal pseudocyst (80% of masses) (28). More than a half of children under the age of two have a high temperature, which is not typical for other age groups.

There is no single definitive diagnostic test available. Elevated amylase/lipase levels are helpful in the diagnosis, although normal serum amylase/lipase levels do not exclude pancreatitis from the differential diagnostic opportunities. The degree of serum amylase/lipase elevation does not correlate with the severity of the disease. Serum amylase level may be normal in the first 24 hours; peak between 24 and 72 hours and in uncomplicated cases, remain elevated for 2 to 5 days. Serum lipase levels are usually elevated in pancreatitis in 1 – 2 hours and remain elevated for longer time than amylase (~7 days). In addition, peak lipase levels were about 5 times higher than amylase levels for the children who have an acute pancreatitis. However, it does not mean that the amylase test is dispensable, because about 10% of patients who have an acute pancreatitis had only elevated amylase. Lipase is elevated in 100 % of patients for children under the age of two, but amylase was elevated in only about 40% to 60%. This discrepancy may be attributed to developmental

differences in the expression of the pancreatic enzymes during the first few months of life (12; 20). Both enzymes may be elevated in cases of other illnesses as well. Hyperamylasemia or hyperamylasuria may be caused by salivary inflammation or trauma; intestinal disease including perforation, ischemia, necrosis, inflammation or result from reduced renal clearance. Other laboratory abnormalities that may be present in an acute pancreatitis include hemoconcentration, coagulopathy, leukocytosis, hyperglycemia, glucosuria, hypocalcemia, elevated γ-glutamyl transpeptidase and hyperbilirubinemia.

Imaging of the abdomen is important as a part of the evaluation of the patient who has an abdominal trauma. Plain abdominal radiographs may reveal an isolated loop of intestine in the vicinity of the inflamed pancreas, the so-called "sentinel loop" for the patient who has pancreatitis Other findings, suggesting pancreatitis, include local spasm of the transverse colon with proximal dilation known as the "colon cut-off" sign. Plain chest roentgenograms should be performed for all patients who have an acute pancreatitis to look for evidence of pleural effusion and pulmonary edema. US is the most useful investigation and may show an increased pancreatic size and a decreased echogenicity. It may also show a peripancreatic fluid collection. It does not subject children to ionizing radiation and it is widely available. The 2 main disadvantages of ultrasound are that it is operator- dependent and an overlaying bowel gas or an obese abdomen can obscure the pancreas. Contrast enhanced dynamic CT scan is the imaging modality of choice: it shows the size, the texture of the inflamed pancreas, a peripancreatic fluid collection, abscess, a pseudo cyst, a ductal dilatation, calcification and perfusion defects or the pancreas to differentiate interstitial pancreatitis from a necrotizing pancreatitis. If in abdominal trauma CT is performed early (< 12 hours after the trauma), the obtained results can be insufficient because the time is needed to visualize the changes of the soft tissues (26; 30). If necessary, CT scan can also be used for interventional procedures for the diagnosis or the drainage of fluid collections. CT is generally recommended to evaluate pancreatitis for several days (72 hours) into the diagnosis when a pancreatic necrosis is suspected clinically. Some research make doubt about the efficiency of computer tomography in diagnostics of the children pancreatitis indicating only 60% of sensitivity and stressing the risk of radiation (12, 20, 29). The practise of choosing US over CT is reasonable for the children till 2 years of age because there is a greater risk of long-term complications with ionizing radiation for young children (5). MR and ERCP have become more essential in the diagnostics and treatment of PTRP because they provide information about the condition of the pancreatic duct. MR reduces children exposure to iniozing radiation. ERCP is useful in the treatment of pancreas pseudocysts, but it disposes patients to the risk of increasing morbidity (pancreatitis 3-14 %, intra-abdominal fluid collection infection risk 10 %) (6, 24).

Several scoring systems are used for adults, but there is no a universally accepted scoring system for predicting severity for children acute pancreatitis. DeBanto et al. developed a new scoring system for children, using age (<7 years), weight (<23 kg), admission WBC count (> 18,500), admission LDH (>2000 IU/L), 48-h trough Ca 2+, 48-h trough albumin, 48-h fluid sequestration, and 48-h rise in blood urea nitrogen. If each criterion is assigned a value of 1 point, then the outcome of patients, with 0-2 points was 8,6 % severe and 1,4 % mortality; with 2-4 points, 38,5% severe and 5,8 % mortality; and with 5-7 points, 80 % severe and 10% mortality. They showed a better sensitivity versus Ranson and Glasgow scores (70% vs 30% and 35%, respectively) and a better negative predictive value (91% vs 85% and 85%) (33). A pleural effusion is highly specific in predicting a severe pancreatitis. The severity of the acute pancreatitis may increase obesity.

**Management**. PTRP treatment among children is a complex measure where the age of children, injuries, caused by the trauma and possible damages of other organs, SIRS, MODS have to be taken into consideration. They have to be placed in the intensive therapy unit, the tests of blood gases, serum electrolytes, creatinine, calcium, glucose intake and output, sometimes also central venous pressure monitoring have to be done. If the severity of the trauma requires a surgical treatment or it has made due to the doubts of the diagnosis, the tissues of pancreas have to be treated maximally carefully. Hemostasis of the pancreas tissues by coagulation has to be abstained; it has to be done by absorptive 5-0 monofilament stitches. Although the efficiency of octreotid application is still being discussed, we tend to apply it in the cases when we perform a direct impact on pancreases tissues. We consider that reduction of pancreatic secretion can decrease the risk of pancreatic fistula development or facilitate their faster closure.

A supportive care with a particular emphasis on the measures that prevent hypoxemia and an insure adequacy of fluid resuscitation is a critical component in the care of patients who have an acute pancreatitis. It is recommended that supplemental oxygen should be administered during the first 24 – 48 hours, especially if narcotic agents are used to control the pain. A supplemental oxygen should be continued until the clinician is fully satisfied that there is no further threat of hypoxemia. The key features in treating of the patients who have an acute pancreatitis are aggressive fluid replacement with crystalloid solution, preferably physiological saline solution (for its higher osmolarity when compared to Ringer Lactate), in aliquots of 20 mL/kg every 20 -30 minutes, with frequent subsequent evaluations, until the signs of dehydration are reversed at the physical exam and parameters such as diuresis (above 1 mL/kg/hour), the heart rate, a pulse and a capillary refill are normalized. Hypovolemia caused by the third space losses, vomiting, diaphoresis, and a greater vascular permeability compromises the microcirculation of the pancreas and is a major contributor to the development of the necrotizing pancreatitis. The second

important consequence of hypovolemia is an intestinal ischemia. There is an evidence that ischemia increases an intestinal permeability to bacteria, the products of bacteria, and endotoxins. The translocation of bacteria is an important cause of the secondary pancreatic infection. The translocation of bacterial products and endotoxins are also potent stimulants of the cytokine release and increases in a nitric oxide that contribute both to an ongoing pancreatic injury and also to the organ failure (particularly respiratory failure) (1). The parenteral analgesics should be started even in a mild pancreatitis because pain can be extreme. Opiods may be necessary; however, the use of maximum doses of medicaments should be avoided, due to the occurrence of nausea and vomiting, besides the contraction of the digestive sphincters. Tramadol can be used at 1 mg/kg/ dose up to four times a day, diluted in a saline solution and in minimum infusion duration of 20 minutes. Nutrition is critically important for the patients who have pancreatitis. Nasogastric suction is only used for the patients who have ileus or a severe vomiting, for a symptomatic relief. Most published guidelines recommend jejunal feeding for 1 to 2 days after the developing of the severe pancreatitis, but there may be a role for slow, continuous nasogastric tube feedings. The total parenteral nutrition is generally not recommended unless a patient proves intolerant to enteral feeding. The studies have shown that some antibiotics like cefuroxime, ciprofloxacin and imipenem cross a blood pancreatic barrier and are found in high level in pancreas and within the necrotic pancreatic tissue (19; 23). It has been shown that in severe acute pancreatitis, i.v. antibiotic prophylactic therapy for 10 – 14 days can reduce the incidence of septic complications. Surgery is indicated if diagnosis is doubt, if patient develops infected necrosis or in sterile necrotising pancreatitis; if patient deteriorates in spite of adequate medical management or if there is pancreatic abscess. An infected pancreatic necrosis needs debridement and a multiple drainage of lesser sac.

In children, only a small percentage of patients were reported to have severe complications, as opposed to adults. A multiorgan dysfunction or a pancreatic necrosis went on to develop for fewer than 6% of children. Early –onset complications primarily include multi-organ dysfunction or shock. Two major organs involved are the lungs and kidneys. An acute respiratory distress syndrome, pneumonia, or pulmonary effusion can develop for a patient. A renal failure has also been observed. Pseudo-cysts were formed for 10 % to 20 % most often associated with a traumatic etiology. Small pseudo-cysts not causing any symptoms can be managed conservatively. Most pseudo-cysts resolve over the course of six weeks. Pseudo-cysts that persist beyond six weeks, those larger than six centimetres, required some degree of surgical intervention. Mortality of acute pancreatitis for children ranged from 0% to 11 %. The reasons for a lower rate of death among children than among adults may include the virtual absence of an alcoholic pancreatitis, an etiology known to carry a much higher rate of mortality. In addition, adults may have lost important protective mechanisms with age that children have retained. Discovering of these potential protective mechanisms may help to elucidate treatments for both adult and paediatric patients with pancreatitis (2).

## CONCLUSIONS

The frequency of PTRP among children tends to increase. It is related to the increase of traumas.

The diagnostics and treatment of children PTRP is complicated. The age group of children under the age of two has to be distinguished as being very different from older children and adults. The early diagnosis and the proper management can contribute to better outcomes for patients and prevent immediate and late related complications.

Children PTRP becomes complicate more frequently than an acute pancreatitis of other etiology by formation of pseudo-cysts, which can resorb spontaneously, it can be successfully treated by a transcutaneous drainage controlled by US or CT or an endoscopic drain to the stomach.

Children who have a pancreas trauma and PTRP have to be concentrated in specialized clinics. A high quality care, a constant monitoring of experienced gastroenterologists and children surgeons have to be ensured in the children intensive therapy unit.

In order to improve the diagnostics and treatment of children PTRP and precisely adapt the adult algorithms for the children in corresponding age groups, it is necessary to have a prospective international cooperation of multicenters.

**Conflict of interest:** None

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10.2478/v10163-012-0014-8

ACTA CHIRURGICA LATVIENSIS • 2012 (12)

*PROBLEM-SOLVING ARTICLE*

# The Assessment of Speech Quality and Intellibility After Replacement of Lost Teeth With Removable Dentures: Review of Literature

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**Summary**
The prevalence of edentulism among Latvian population is high. According to the World Health Organization criteria complete tooth loss is regarded as a disability. One of the basic oral cavity functions is taking part in speech production. Alterations in oral cavity, caused by tooth loss and resorption of its supporting alveolar ridge, can produce changes in speech quality and intelligibility. This can lead to psychological and social problems, thus decreasing the patient's quality of life. There are several options in replacing missing teeth. In Latvia prosthetic rehabilitation with removable dentures is in great demand. It is mentioned in literature, that removable dentures improve speech quality and intelligibility. However, there is no consensus in this field. The objective of this review is to screen literature for data related to the assessment of speech quality and intelligibility after replacement of lost teeth with removable dentures. Literature was selected through several electronic databases, as well as a manual search in dental journals. The detailed analysis of thirty two articles was performed and some significant facts were revealed. There exists an opinion, that removable dentures may have a negative effect on speech production. As the volume of oral cavity is diminished, resonation of oral cavity is decreased. Artificial teeth, removable denture base can limit tongue space and change the location of its articulation contacts. The assessment of speech quality and intelligibility is an essential diagnostic tool before and after prosthetic rehabilitation in order to provide the best option for restoring lost function. A standardized method for determining changes in speech quality after tooth loss and further prosthodontics has not yet been established. Semi-standardized instruments for the analysis of speech disorders are well documented when dealing with speech changes before and after prosthetic rehabilitation with various oral osseointegrated implant supported prostheses. For the Latvian language this method has not been described yet.
**Key words:** tooth loss, edentulism, denture, speech quality and speech intelligibility

## INTRODUCTION

The prevalence of edentulism among Latvian population is quite high (19, 40, 41, 42). Despite the fact that the average age of becoming edentulous is increasing, the total number of edentulous patients is declining less rapidly than could be expected (7, 33, 40, 42). This can be explained by increasing life expectancy. Therefore, 27.17% of Latvian inhabitants at the age of 35-44 require prosthetic rehabilitation and at 65-74 years of age oral rehabilitation is needed for 60.4% (19, 21, 22). It is estimated (8) that the USA adult population in need of 1 or 2 complete dentures will increase from 33.6 million adults in 1991 to 37.9 million adults in 2020. According to World Health Organization (WHO) criteria (44) an individual who has lost all teeth, can be compared to a person who has lost a whole part of his body, and is considered to be physically impaired (21, 42, 44). Tooth loss causes chronic disability; many edentulous patients have difficulty performing essential tasks such as eating, speaking and socializing. Teeth are important from the point of view of esthetics and their loss leads to the patient's dissatisfaction. Some patients may experience increased social and psychological problems (22, 40). One of the basic oral cavity functions is speech production (9, 12, 45). Speech is an important form of verbal communication in society, which directly and indirectly affects person's quality of life (5, 7, 12, 27). Its disorder can interfere with the daily and professional activities, especially in such professions as teachers, actors, singers, aviators (30). Rothman (32) states that normal speech production depends on proper functioning of five essential mechanisms: 1) motor, consisting of the lungs and associated musculature, 2) vibrator – vocal cords, 3) resonator, consisting of oral, nasal, pharyngeal cavities and paranasal sinuses, 4) initiator, consisting of the motor speech area of the brain and 5) articulators. Articulators can be divided into static or passive (teeth, hard palate, and alveolar ridge) and dynamic or active (tongue, lips, soft palate or velum) components (4, 35, 45). Teeth and the above mentioned structures take part in performing the functions of oral cavity such as mastication and speech production. They play an important role in articulation of many speech sounds (14, 31, 37, 45). Alterations in oral cavity caused by tooth loss and resorption of alveolar ridge can produce changes in speech quality and intelligibility. In general, two main elements are influenced by these alterations – the place of articulation of the tongue with hard palate and alveolar ridge mucosa changes (12, 28, 35, 37) and the resonator function of the oral cavity decreases (12,

22, 27). Each language has its own phonetic system (25) that is why the assessment of speech quality and intelligibility after tooth loss and its replacement with removable dentures should be performed in the patient's native language (25, 45). This type of study has been made for German (17, 34, 35), Czech (16), Italian (10), Turkish (27), Croatian (38) and Japanese (43) languages. In Latvian literary language there are 48 sounds (12 vowels, 10 diphthongs, 26 consonants) (25), as compared with 44 sounds in English (19 vowels, 25 consonants) (30, 45), the Czech language has 40 sounds (10 vowels, 3 diphthongs, 27 consonants) (16). When producing Latvian vowels the tongue and lips are performing their corresponding resonators (5, 32). Tooth loss and its consequences and further prosthetic rehabilitation do not usually influence vowel production (45). However consonants are usually affected (5, 9, 12, 31, 35). Speech pathology uses the term "dyslalia" which means speech sound production disorders in the native language in conditions of normal hearing and speech organs innervation (25). Clinical cases analysis indicates (9, 10, 12, 14, 28, 31, 34, 35, 37, 45), that tooth loss and prosthetic rehabilitation with removable dentures most often affect sibilants (S, Z). In speech pathology this type of distorted pronunciation is called "sigmatism" (25, 12). Patients call this defect "lisping" (10, 32). More than 90% of Latvian consonants are produced by the tongue articulating with teeth, hard palate and anterior part of the alveolar ridge (5, 16, 25). Physiologically consonants are characterized as sounds or noises which occur when the exhaled air flow overcomes the obstacle (5, 25). Depending on air flow obstruction place the Latvian language consonants are divided into bilabial (p, b, m), labiodental (f, v), dental (t, d, s, z, c, dz, n), alveolar (l, r), alveopalatal (š, ž, č, dž), palatal (ķ, ģ, j, ņ, ļ), velar consonants (k, g) and pharyngeal (h) (5, 12). The aim of oral prosthetic rehabilitation (45, 46) is to restore oral cavity functions – food milling, speech, comfort and esthetics, thus improving the patient's quality of life and self- esteem (14, 31, 42, 45, 46, 39). In case of extensive or complete tooth loss, it is possible to fabricate acrylic removable dentures, supported by soft tissues. Such constructions are known to have several shortcomings - they cover a large part of prosthetic field, their functional value depends on oral anatomy and they are removable (13, 28, 45). It is assumed that food milling capability with removable dentures is restored to 25% of the total masticatory effectiveness (33). The restoration of oral function plays an important role in patient's satisfaction with removable dentures and oral rehabilitation acceptance (7, 33, 37, 38, 46). Despite all limitation, the degree of patient satisfaction with removable partial and total dentures is high (7, 33). In Latvia this type of restoration of tooth loss and its supporting alveolar bone is in great demand (19, 40, 41, 42).

**The influence of removable denture on speech quality and intelligibility**

It is mentioned in literature (13, 14, 17, 28, 38, 39), that removable dentures improve speech quality and intelligibility. However there's no consensus in this field. It is considered (9, 10, 13, 28, 31, 38, 45), that removable dentures may have a negative effect on speech production, as the volume of oral cavity is diminished. Artificial teeth, removable denture base can limit space for the tongue and change location of its articulation contacts with hard palate and alveolar ridge mucosa (5, 24, 28). Nowadays, a number of methods are available for speech assessment. Some of them are more useful for speech changes analysis in case of tooth and supporting structure loss and after prosthetic rehabilitation with removable dentures (16, 17, 28, 31, 34, 35, 37, 45). A standardized method for determining speech quality changes after tooth loss and further prosthodontics has not yet been established (17, 23, 37). Semi-standardized instruments for the analysis of speech disorders are well documented when dealing with speech changes before and after prosthetic rehabilitation with various oral osseointegrated implant supported prostheses (24, 23). Two facets of speech perception are described in literature – speech intelligibility (17, 31, 37) and speech quality (16, 28, 34, 35). Both of them are multidimensional terms. Speech intelligibility is defined as ability of a native educated listener to perceive oral speech, understand it, and distinguish separate sentences, words, and letters (37, 39). One of the most popular analyses of speech quality and intelligibility is evaluation done by speech pathology (12, 27, 31). For more reliable results several experts' evaluation is required. In order to perform speech analysis direct speech samples or audio or video records, made in accordance with standardized speech pathologist tests, can be used (16, 24, 31). Rodrigues published analysis of speech in different oral prosthetic rehabilitation modalities for elderly individuals. Video speech samples of 36 patients were analyzed by five speech-language pathologists. It was concluded that individuals using removable dentures present alteration in linguodental and alveolar phonemes. The type of prosthesis and its stability do not seem to interfere with speech production (31). Hassel proposed an easy method for improving speech function of complete maxillary denture (two speech therapists participated in this research) (10). In his turn, Ozbek evaluated the articulation of Turkish phonemes after removable denture application, 3 speech pathologists investigated the speech records of 15 patients before and after removable denture insertion and after a week of adaptation. The results showed that for some phonemes, problems in articulation occurred after the insertion of a removable partial denture while for others a significant amelioration was observed. In general, problems in articulation of evaluated phonemes were resolved after one week of adaptation (11). The speech pathologist's analysis is considered to be "golden standard" in case of prosthetic rehabilitation (12, 17, 37). However, this method has some drawbacks, as its objectivity depends on the expert's experience, acute of hearing and, perhaps, psychological perception (37). It is essentially a subjective expert investigation method,

so researchers are looking for more objective methods of speech changes qualitative analysis. A group of German specialists introduced and validated a computer-based speech recognition system for the standardized and automatic speech assessment in edentulous patients after dental rehabilitation with complete removable denture (17, 37). With the help of this system the speech intelligibility and word accuracy (WA) can be measured. Stelzle (37) applied this system to the analysis of speech changes after tooth loss and prosthetic rehabilitation with removable dentures in 28 patients. The author came to the conclusion that the system can be used for speech intelligibility evaluation and also confirmed the fact that complete loss of teeth can cause a dramatic deterioration of speech and removable complete dentures do not fully solve the problem. In 2011 using the same system Knipfer (17) analyzed 45 patients and found that removable dentures improve speech intelligibility - after six month of adaptation the quality of speech in control group and the experimental group was equal. Speech quality is associated with the sound volume, intensity, intonation, timbre, sound pronunciation accuracy and physical spectral characteristics (28, 34, 35). The spectral analysis is widely used for quality of speech evaluation (16, 28, 34, 35). It is an independent diagnostic tool for the assessment of speech quality regarding alteration of the dental arch. This is an objective, accurate and reliable method of sound analysis, but it can be used to investigate only certain sound parameters and it is not possible to make evaluation of the overall speech quality changes (17, 37). With the help of spectral analysis the acoustic characteristic of speech sound can be determined. There are dynamic and static spectrograms. For speech quality changes evaluation the static spectrogram, namely one obtained by a computer program fast Fourier transformation (FFT) power spectrum, is used more frequently (11, 12, 16, 35). The speech sound is analyzed in three dimensions: frequency, amplitude and chronologic sequence. The "S" sound pronunciation is most often used for spectral analysis (10, 34, 35). Its formation mechanism is complicated (12, 29, 34, 35). In spite of considerable interlanguage phonetic diversity, "S" sound pronunciation mechanism is similar in most languages (34, 45).

**The arrangement of artificial teeth and speech sounds production mechanism**

Spectral analysis is usually used to measure the influence of the maxillary central incisors on speech quality changes before and after prosthetic treatment (28, 34, 35). Stojevci (38) analyzed the formation mechanisms of Croatian dental and postalveolar group of sounds and removable denture impact on the sound pronunciation accuracy. The results showed that subjects with partial removable denture had 50% less distortion variables and that prostheses did not completely restore articulation of postalveolar sounds. Groups with and without removable dentures had lower formant peaks intensity and wider formant bandwidths as compared with the control group. Partial removable dentures have not significantly interfered with resonance frequency. At the same time, pronunciation of the examined sounds was significantly improved; however, precision of the articulation movements has deteriorated (38). Using the spectral analysis method Runte indicates that the maxillary incisors play an important role in the "S" sound production and even minimal displacement of the teeth, causes the sound production distortion. The labial angulation seemed to have a greater effect than the palatal displacement (34, 35). It was also concluded that neuromuscular reactions are more important for initial speech sound distortions than are aerodynamic changes in the anterior speech sound - producing area (34, 35, 45). That is why artificial teeth should be placed in the original position of the lost natural teeth (10, 34, 35). For artificial teeth arrangement it is recommended to use anatomical landmarks (papilla incisiva and first prominent rugae palatinae), these will ensure the artificial teeth arrangement in agreement with phonetic and esthetic requirements (35, 45). In production of sibilant sound "S" lower jaw protrudes and takes the position that corresponds to the "closest speaking space" (CSS), which means that 1-2 mm gap is formed between upper and lower incisors (29, 32). It is assumed that the distance is constant and doesn't change during lifetime; however there is no confirmation of this fact (it is stated in literature, that parameters vary from 0 to 10 mm) (6, 36). In the process of production of "S" sound the lateral surface of the tongue occludes with the upper premolars and its supporting alveolar ridge, the exhaled air flow is pushed between the front teeth and thus characteristic noise is formed (29, 30). An essential factor in the production of correct "S" is the proper grooving of the tongue (9, 29, 32). In case this groove is too shallow, "S" sound is softened and becomes similar to "Sh" sound, that's why if the removable dentures base is thicker in the anterior part the lisp distortion occurs (9, 18, 32, 33). If this groove is formed too deeply, the patient will whistle and then it is recommended to make the removable dentures base thicker (9, 18, 32). "S" sounds production can be distorted as a result of air leakage, when the tongue is not sufficiently supported in the bicuspid region (1, 32). In addition to spectral analysis, it is possible to use palatogram (4, 35). This method is suitable for identification of the exact contact of the tongue with hard palate. The palatogram is made by covering the palatal surface of existing denture with colorant. Then during pronunciation of the tested phonemes the exact places of the tongue and denture base contact can be seen. Several methods of palatography are described in literature (3, 4, 9, 18), but the purpose is the same – to personalize removable denture base to patient's physiology, so that quicker phonetic adaptation can be achieved (9, 18, 29, 32). In order to check and verify the artificial anterior incisor position and length it is proposed to use "F" and "V" phonemes tests. When producing these sounds the upper incisors are touching the lower lip at the Vermillion border or dry-wet line (45). If teeth are too short, "V" will sound as "F" and if

teeth are too long "F" will sound as "V" (12, 32, 33, 45).

**Removable denture base and speech sounds production**

In removable denture the base is an essential part of the construction (5, 27, 28, 45). It provides retention, support and stability and at the same time it can cause significant phonetic distortions (9, 13, 18, 28). In case of complete removable denture its margins have to be maximal in order to provide its functionality (30, 32, 45). If margins are too short, the functional value of the construction will be decreased, on the other hand if margins are too long bilabial sounds (p, b, m) will be distorted, because the lips will not occlude properly (30, 32, 45). The distal margin of removable denture plays a significant role – a properly placed distal margin has to cover fovea palatine and to be situated on "A" line. It is recommended to use "A" sound phonetic test (30, 32). Correctly placed distal margin will decrease the air flow under the denture, which will improve the functional value of the denture and will minimize phonetic abnormality (2, 30, 32). When planning removable partial denture design, it is advisable to avoid covering of hard palate in anterior part, as in 90% of consonants production the tongue articulates with this region (9, 12, 32). While modeling the removable denture base it is essential to preserve hard palate configuration and avoid unnecessary reduction of oral cavity volume (9, 18, 28). The denture has to be well adapted to the oral cavity peculiar anatomy features (28). Denture base should be as thin as possible in order to minimize alteration of oral cavity resonance (5, 28, 29), as well as to minimize the distortion of tongue movement during speech sound production. At the same time the removable denture should be thick enough to withstand its deformation under masticatory forces (45). It is considered that (5, 13, 28) the optimal thickness of the denture base should be 1.4-2 mm thick. Rugae reproduction on removable dentures base is a subject of significant controversies (28, 32). On the one hand it will make denture base thicker, on the other hand there are certain sounds, namely palatolingual „T", „D", „N", and „L" for whose production tongue orientation elements are needed (12, 32). In case of metal removable denture the major connection is made by using corrugated surface, therefore reducing the "foreign body sensation" and providing faster phonetic adaptation (9, 10, 28).

**Phonetic adaptation of patients with removable denture**

The phonetic adaptation of a patient with removable dentures depends on: the patient's individual adaptation capacity (16, 27, 29, 34, 45), his sound recognition capacity, sharpness of hearing (24, 45), functional value of the dentures (5, 14, 24, 45), artificial teeth selection and arrangement (16, 29, 34, 35, 45), removable dentures base design and thickness (4, 16, 43, 45). Speech is an acquired complex process (27, 45). After removable denture insertion the oral cavities volumetric parameter alters and as a result transitory speech distortion occurs (16, 17, 28, 45). According to the research data phonetic adaptation to the removable partial denture lasts from 2 to 4 weeks (28, 31, 45). As for senior patients their individual adaptation capacity decreases, and speech function restoration may take longer time, which is usually connected with deterioration of hearing and overall aging process (13, 45). It is recommended to warn the patients before insertion of new dentures about the possible speech problems and to provide information on the possibility of improving of speech quality by doing some exercises and practicing difficult phonemes (45). It is generally recognized (27, 35, 45) that artificial teeth in dentures should be placed in lost teeth places, in order to match the patient's existing neuromuscular pattern. It will ensure faster phonetic adaptation because in this case fewer adjustments in patient's neuromuscular pattern are needed. The less difference there is between the form, position, palate topography, configuration and anatomy of the lost teeth and the restored artificial ones, the quicker and easier the phonetic function will be restored (the theory of "less effort") (45). If changes in oral cavity are great and are beyond the patient's individual adaptation capacity, persistent speech disorder will occur (22, 38, 45).

**The functional value of removable dentures (support, retention, stability)**

As well as all factors mentioned above, the functional value of removable dentures plays an important role in speech production (22, 39, 45). The tongue of patient wearing denture has an additional function of stabilizing and retaining the denture. With diminishing functional value of the prosthesis, more of additional function of the tongue is required; it may have negative impact on speech quality (22, 30, 39). The functional value of removable dentures depends on the precision of its production. Inaccurate fabrication of denture will produce a defective prosthesis, which doesn't correspond to the prosthetic field and thus its functional value is decreased (30, 39, 45). Even if after insertion of removable dentures they match the functional requirement, after some period of exploitation there functional value can be reduced (39, 45). The denture can become loose and disturbing to the patient. This can be caused by alveolar bone resorption, which is a physiological process after tooth extraction (15, 20, 22). The process is especially rapid in the course of the first year after tooth extraction; as a result the volume of alveolar bone decreases and its morphology changes. The total supporting surface is reduced forming an unfavorable prosthetic field (15, 20, 22). Recently much attention has been paid to atraumatic tooth extraction and effective bone and soft tissues preservation procedure (15, 20). The method of tooth extraction influences the degree of consequent bone resorption. Further prosthetic rehabilitation success, both functional and esthetic, depends on preservation of the remaining structure morphology and quantity (15, 30, 45). Several methods (15, 20) for alveolar ridge preservation after tooth extraction have been reviewed in literature; however, due to diversity of research design it is impossible to conclude which is the

most effective one. Thus the problem of soft and hard tissues preservation after tooth extraction remains so far unresolved. After tooth extraction and immediate prosthetic treatment the patient should be informed of the necessity of further corrections, relining and controlling of removable denture (45).

## CONCLUSIONS

Analysis of the data found in current dental literature has made it possible come to the following conclusions:

- Alterations in oral cavity caused by tooth loss and resorption of alveolar ridge can produce changes in speech quality and intelligibility.
- Rationally planned and designed removable denture, made according to phonetic needs, improves patient's speech function.
- Removable dentures functional value is an important factor for restoration of the lost function, including speech.
- The degree of resorption of the bone is one of decisive factors for successful outcome of oral rehabilitation. Tooth extraction methods and techniques partially influence the degree of further bone resorption.

**Conflict of interest:** None

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10.2478/v10163-012-0015-7

ACTA CHIRURGICA LATVIENSIS • 2012 (12)

*CASE REPORT*

# Recurrent Gallstone Ileus in an Elderly Patient With Significant Co-Morbidity

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**Summary**
Gallstone ileus accounts for 1-4% of mechanical bowel obstruction. As the patients are elderly, significant co-morbidity is frequent, therefore early diagnosis and timely surgical treatment is mandatory to prevent patient's death. The aim of the present case report is to heighten the awareness of surgeons to the occurrence and differential diagnostics of gallstone ileus. Here we describe recurrent intestinal obstruction in an elderly lady with previously treated peritonitis as well as history of hip joint prosthesis and mitral valve replacement.
**Key words:** calculous cholecystitis, cholecystoduodenal fistula, gallstone ileus, Bouveret syndrome, peritonitis

## AIM OF THE DEMONSTRATION

Aim of the demonstration is to describe a rare surgical pathology in order to heighten the awareness of surgeons so that gallstone ileus can be included in the differential diagnostics of intestinal and gastric outlet obstruction.

## CASE REPORT

Seventy-two-year-old female was urgently admitted to Clinical University hospital with severe pain in the umbilical region. The pain had lasted for 2 days. Following the onset of pain, bouts of vomiting had developed as well. Physical investigation revealed severe pain, abdominal wall rigidity and rebound pain by palpation. Intestinal peristalsis was absent. There were signs of deep dehydration. The patient had complicated medical history including 2 previous urgent hospital admissions within several months. Four months before the present episode, she had had prolonged abdominal pain. Destructive calculous cholecystitis was diagnosed. By laparoscopic approach, a perivesicular infiltrate was drained. Three months later, the patient was admitted to the hospital with severe abdominal pain. Obstructive ileus was revealed. By laparotomy approach, a stone-like object was removed from the intestines through enterotomy. Surgeon interpreted this object as a phitobezoar. In all previous cases, the surgical treatment was embarrassed by overdosed anticoagulant therapy due to hip joint and mitral valve prostheses. The hypocoagulation was corrected by fresh frozen plasma.
Taking into account the patient's medical history, urgent computed tomography (CT) of abdomen with oral and intravenous contrast enhancement was performed. By CT, infiltrate and abscess-like cavity measuring 3 cm was found in the subhepatic area (Fig.1). Exudate was found in the right abdominal flank and in pelvis. There were signs of ileus in small intestines as well (Fig.2). Laboratory findings showed elevated C-reactive protein of 244.9 mg/L [laboratory reference interval 0-5.0 mg/L], creatinine 118 mkmol/L [35-97 mkmol/L], urea 10.2 mmol/L [2.0-7.1 mmol/L], direct bilirubin 11 mkmol/L [<7 mkmol/L], ASAT 55 U/I [<41 U/I] and ALAT 58 U/I [<58 U/I]. The level of alpha amylase in the urine was 1094 U/I [<460 U/I]. Anticoagulants have been used before admission to the hospital, so patient had coagulation disorders reflected by the following findings: activated partial thromboplastin time 37.6 sec., international normalized ratio (INR) 2.5, fibrinogen 4.4 g/L and prothrombin index 25.4%. The white blood cell count was $4.0x10^9$/L; platelet count $125x10^9$/L and red blood cell count $3.52x10^{12}$/L.
Based on clinical findings and imaging, ileus was treated conservatively for 1 day. As there was no positive dynamics, surgical therapy was applied. During laparotomy, diffuse stercoral peritonitis was found with fibrin-rich exudate. It was caused by hemicircular intestinal perforation located 160 cm proximally from ileocaecal valve, just before the previous enterotomy scar. A stone was impacted in the perforation. After the intestinal integrity was repaired, inspection of abdominal cavity disclosed 3 cm wide cholecystoduodenal fistula with perforation (Fig.3). The fistula was closed as well.
Postoperatively patient was treated in intensive care unit for 3 days. Later, reaching haemodynamical stability, she was transferred to surgical department. Postoperative recovery was long and slow. Vancomycin was administered to eradicate methicillin-resistant *Stapholococcus aureus*. Hydrothorax was eliminated by puncture. After massive medical treatment for 5 weeks, eventually patient was discharged with improvement. No recurrences of peritonitis or acute abdominal pain of any reason have occurred during the following 5 months.

## DISCUSSION

Calculous cholecystitis is a frequent disease in Western world. The total amount of stones in the gall bladders in the population is estimated in tonnes. The presence of gallstones can lead to diverse complications. Biliary enteric fistula develops in 2-3% of patients with cholecystolithiasis and recurrent cholecystitis (Roslyn *et al*., 1987; Giese *et al*., 2010). If choledochoduodenal fistula has developed, the stones can pass into the small intestines and occasionally become trapped causing ileus. Gallstone ileus accounts for 1-4% of mechanical bowel obstruction and in aged persons (older than 65 years) – for 25% of non-strangulated small bowel obstruction cases (Giese *et al*., 2010). Due to the sedimentation of intestinal content, gallstones can increase in diameter and more frequently impact in the distal part of small intestine (Giese *et al*., 2010). The impacted stone is located in the terminal ileum in 50-75%, proximal ileum and jejunum in 20-40%, and in duodenum in 10% cases. If gastric outlet obstruction develops due to gallstone impaction in the duodenum or pylorus, the condition is called Bouveret syndrome. It is a rare manifestation of gallstone ileus, caused mainly by large stone. Until 1999, only 175 cases of Bouveret syndrome were reported (Ariche *et al*., 1999).

In the diagnostics, history of recent gallstone disease exacerbation should alert attention. However, as in our case, the formation of fistula itself can occur during clinically silent period after the exacerbation (Giese *et al*., 2010). CT and ultrasonography can provide valuable information (Giese *et al*, 2010). However, occasionally the findings are pathological still ambiguous. Thus, in our patient cholecystoduodenal fistula was interpreted as an abscess. Presence of stone in the intestinal perforation must prompt intraoperative investigation of biliary system.

The treatment of gallstone ileus is aimed at removal of the obstructing stone. The treatment can include endoscopic methods, especially in case of proximal stone location as in Bouveret syndrome. Endoscopic extraction, extracorporeal shock wave lithotripsy or laser lithotripsy can be successful (Maiss *et al*., 2004; Tanwar *et al*., 2008). However, endoscopic treatment can fail if the stone is large (O'Neill *et al*., 2009). In addition, the fragments can undergo growth by sedimentation resulting in more distal stone impaction and recurrent ileus (O'Neill *et al*., 2009). Regarding surgery, it is recommended to remove the stone through enterotomy using either laparoscopic or open surgical approach, and leave the gallbladder and fistula in situ as this is associated with lower mortality and recurrent complications are rare (O'Neill *et al*., 2009). However, in our case, the course of disease was already notable for recurrences therefore restitution of intestinal integrity was followed by closure of the fistula during the same operation. The outcome was beneficial as the patient survived and experienced no further recurrences. Thus, our experience is more in line with the contrary suggestion of active surgical treatment and closure of fistula (Iancu *et al*., 2008).

Significant co-morbidities are described in gallstone ileus patients, including frequent consequences of atherosclerosis (O'Neill *et al*., 2009). The association can be attributed to common occurrence of both cholecystolithiasis and atherosclerotic heart and vessel disease in elderly group. In contrast, vascular pathology could enhance ischemic changes thus facilitating fistula formation. Regardless of pathogenesis, this association compromise the outcome and can become cause of death. Therefore the diagnosis should be reached early, before peritonitis has developed.

**Conflict of interest:** None

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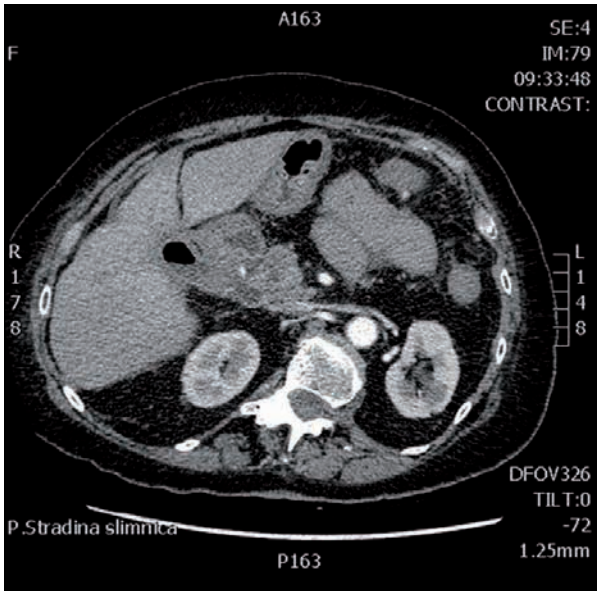


**Fig. 1. Abdominal computed tomography findings before the operation. Note the subhepatic infiltrate and cavity.**

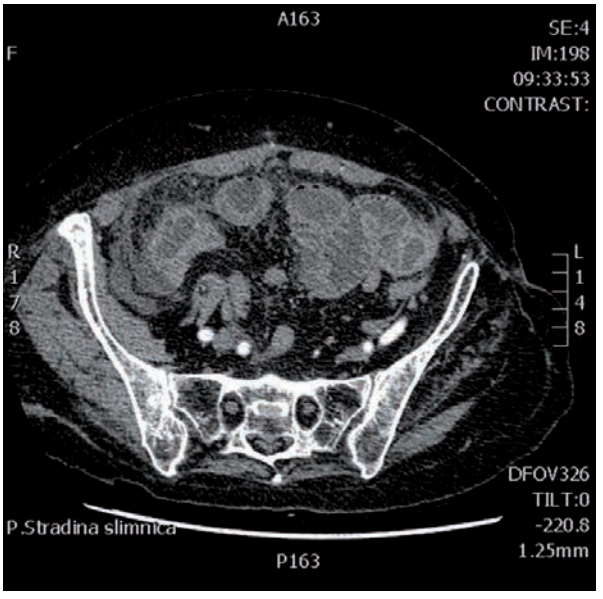


**Fig. 2. Abdominal computed tomography findings before the operation. Note the markedly distended intestinal loops.**

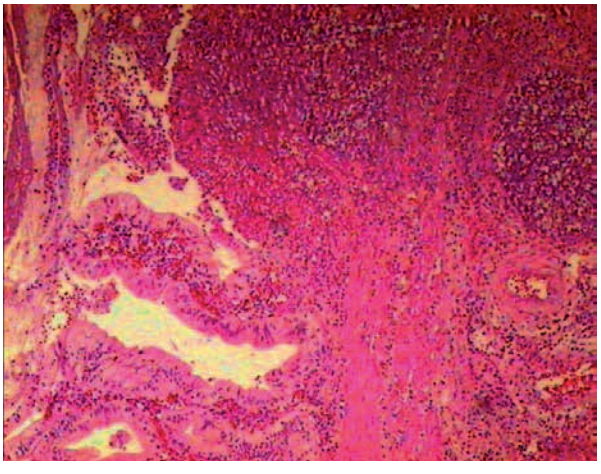

**Fig. 3. Tissue structure of the resected holecystoduodenal fistula. Note the deep necrosis and purulent inflammation in the wall of fistula reflecting changes adjacent to fistula perforation site. Haematoxylin-eosin, original magnification 50x.**

10.2478/v10163-012-0015-7



*CASE REPORT*

# Management of Adenoid Cystic Carcinoma of Distal Trachea

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**Summary**
Tracheal neoplasms occur infrequently, the majority of primary tracheal tumours in adults are malignant, and in 40% of cases it is adenoid cystic carcinoma, which can be asymptomatic for a long period of time. Surgical treatment can be justified even in advanced disease, because progression of tracheal tumour usually causes obstruction of airways. Improved long-term outcome seems to follow combined surgical resection and full-dose radiotherapy. Patients after resection of adenoid cystic carcinoma need careful and long-term observations to detect a relapse.
**Key words:** Adenoid cystic carcinoma, tracheal resection, tracheal obstruction

## AIM OF THE DEMONSRATION

The aim of this demonstration is to show the management of patient with delayed diagnosis of adenoid cystic carcinoma of the distal trachea which caused near-total obstruction of the lumen of trachea.

## CASE REPORT

A 38-year-old female presented to the Emergency Department of Regional Hospital for fever, shortness of breath and non-productive cough in May 2011. A chest X-ray revealed normal findings and CT scan findings were also interpreted as normal, therefore she was diagnosed with chronic obstructive pulmonary disease and sent to local pneumonologist. After prolonged treatment with inhaled corticosteroids and bronchodilators, she still had shortness of breath on exertion and mild wheezing. In September 2011 she was referred to the Department of Pulmonology of our institution. Fiberoptic bronchoscopy revealed approximately 4 cm long circular tumour in the distal part of trachea with partial obstruction of tracheal lumen (Fig.1). Straightforward rigid bronchoscopy was performed for partial resection of tumour tissues, and silicone Dumont stent was inserted to avoid compromising airways (Fig.2). Pathology was consistent with adenoid cystic carcinoma. Following CT scan showed potential resectability and no distant spread of the tumour (Fig.3). Decision to proceed with surgery was made. Standard right lateral thoracotomy approach was chosen. Transthoracic approach provided excellent access to the lower trachea and carina, including the left main bronchus. Intraoperative fiberoptic bronchoscopy was used for accurate tumour localization. Resection of distal part of trachea was performed by using combined cross-field intubation and high-frequency jet ventilation techniques (Fig.4). Anastomosis was made by running non-absorbable monofilament 3-0 suture. Macroscopically complete resection of tumour was achieved (Fig.5). The patient had uneventful recovery after surgery. Unfortunately final pathology results revealed tumour cells in the resection margins, therefore patient was referred for radiotherapy. Patient completed full-dose (60 Gy) radiotherapy course on January 2012. The last follow-up was made one year after surgery in September 2012 and showed no signs of relapse (Fig.6). Long-term follow-up is scheduled for this patient.

## DISCUSSION

The incidence of primary tracheal tumours in the general population is not precisely known, tracheal tumours are accounting for less than 1% of all malignancies[1]. Primary tracheal neoplasms are still often diagnosed long after the onset of symptoms. Tracheal tumours often present with signs of upper airway obstruction. Delay in diagnosis occurs because the pulmonary fields remain normal on a chest radiograph[2]. Adenoid cystic carcinoma of the trachea occurs over a wide age range, from the twenties through the seventies with slight female predominance. No relationship has been discerned with cigarette smoking or other known carcinogenic factors. Adenoid cystic carcinoma seems to be more prevalent in the lower trachea and carina. Stage for stage, adenoid cystic carcinoma has the better prognosis, with 5-year survival rates cited to be 66-100% and 10-year survival rates of 51-62%[3]. Adenoid cystic carcinoma has been reported to have sensitivity to radiation[4].

There is a higher risk for local recurrence and positive surgical margins with distal tracheal location[5], as we described in our case. Late local recurrence of tracheal adenoid cystic carcinoma after apparent cure by surgery and irradiation is a discouraging characteristic, as adenoid cystic carcinoma can invade microscopically submucosally and perineurally for long distances beyond grossly visible disease. Frequently, adjacent to

the main mass of tumour, which clearly projects from the mucosa, there is evidence of tumour infiltration beneath the mucosa or in the tracheal wall, the margins are often indistinct.

Surgery is usually indicated once diagnosis of a primary tracheal neoplasm is made. This is because these patients tend to progress rapidly once symptomatic because of the near-total tracheal luminal obstruction that is frequently present.

Tumours of the lower trachea and carina are best approached through a high right posterolateral thoracotomy. Some surgeons (Pearson) prefer a median sternotomy. Sometimes it may become necessary to accept microscopic tumour at the margins of resection, in order not to compromise the possibility of healing by excessive anastomotic tension following an extended resection[6]. Long-term survival more than 10 years, however, is also observed after tracheal resection of locally advanced adenoid cystic carcinoma[7].

**Conflict of interest:** None

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**Fig. 1. Initial endoscopic (fiberbronchoscopy) view.**

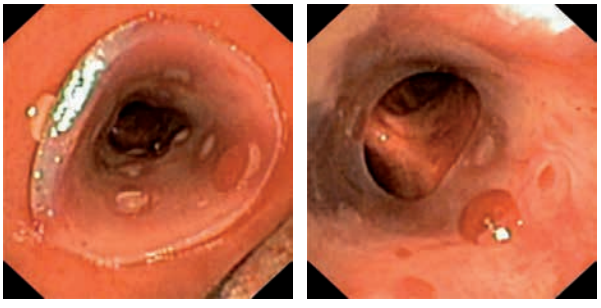


**Fig. 2. Endoscopic view (fiberbronchoscopy) after insertion of silicone Dumont stent.**

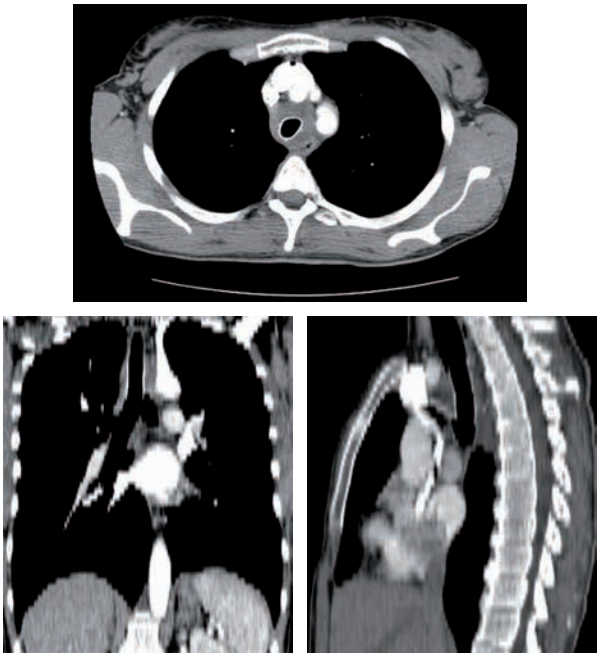
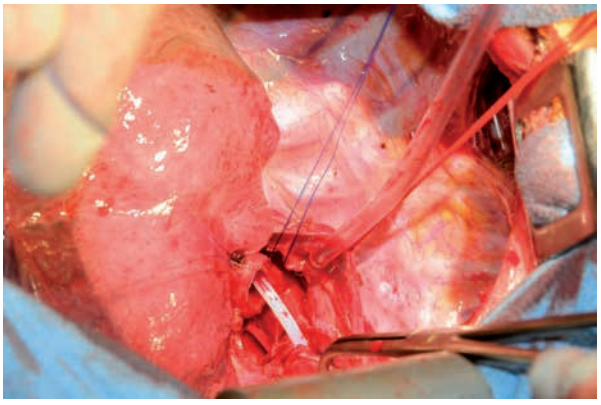
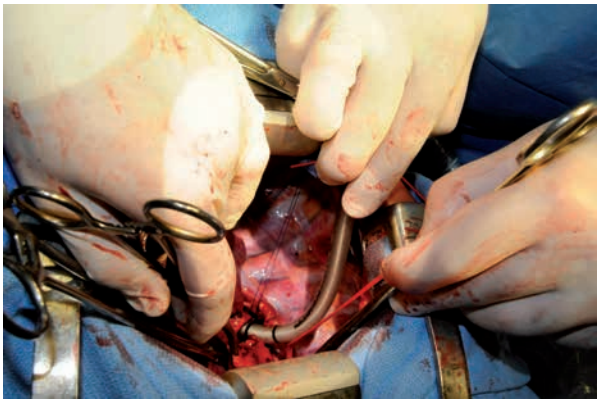


**Fig. 3. CT scan (above) and reconstructions in frontal and sagittal view (below) showing stent in the distal trachea and no signs of distant spread of the tumour.**





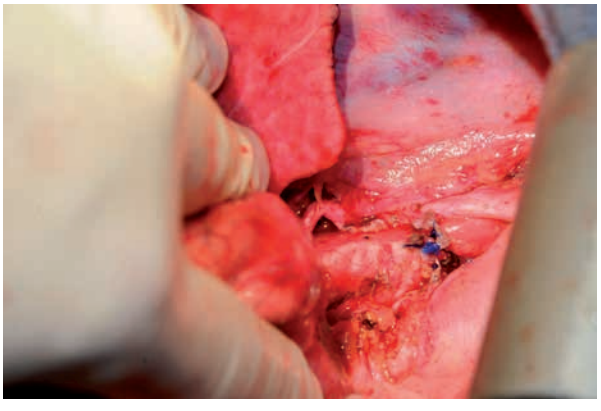


**Fig. 4. Intraoperative views: resection of trachea using high-frequency jet ventilation technique (above), starting anastomosis of posterior wall of trachea using cross-field intubation technique (middle), tracheal anastomosis completed (below).**

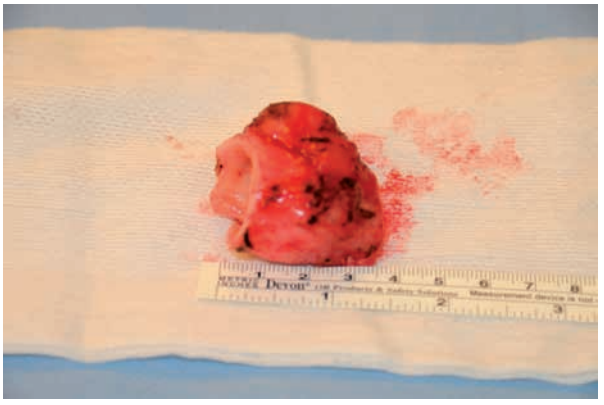


**Fig. 5. The resected specimen.**

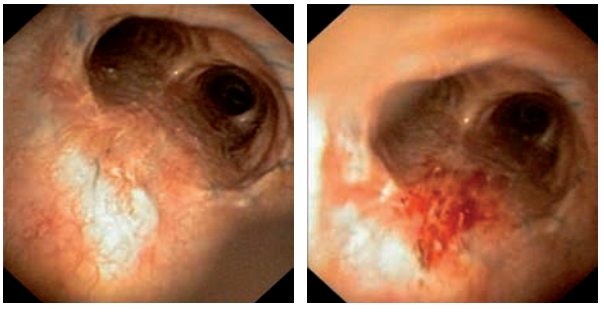


**Fig. 6. Endoscopic view on follow-up one year after operation – before (left) and after (right) control biopsy.**

10.2478/v10163-012-0016-6

ACTA CHIRURGICA LATVIENSIS • 2012 (12)

*CASE REPORT*

# Granular Cell Tumour of the Breast Cancer: Challenging Clinical and Radiological Mimic of Cancer

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**Summary**
Granular cell tumours (GCT) are rare soft tissue neoplasms of unclear histogenesis affecting almost any organ or tissues. Approximately 5% of GCT cases are located in the breast. Although granular cell tumour is benign in most instances, it can mimic breast cancer by radiological and clinical traits. Increased awareness of GCT is necessary to avoid misinterpretation as cancer and subsequent overtreatment.
**Key words:** breast tumour, granular cell tumour, immunohistochemistry

## AIM OF THE DEMONSTRATION

In order to heighten the awareness of surgeons about the differential diagnosis of breast cancer and about the choice of appropriate extent of surgical treatment, we describe here a rare benign tumour resembling breast cancer clinically and radiologically.

## CASE REPORT

Fifty-six-year-old female consulted doctor with complaints about palpable hard mass in her left breast. The mass was located in upper inner quadrant. By mammography (MG), a spiculated lesion was evident (Fig.1). Core biopsies were obtained under ultrasonography (US) guidance. Biopsy investigation yielded granular cell tumour with invasive growth. Surgical treatment was recommended. Extended sectoral resection with axillary lymph node dissection was performed. The postoperative period was uneventful.

On gross examination of the removed breast tissues, there was solid, gray, firm mass, measuring 1.7x1x0.8 cm. The gross structure suggested invasive growth due to radiating margins. By histology, the tumour was composed of large cells with amphophilic, finely granular cytoplasm, small dark nuclei and thus low nucleo-cytoplasmic ratio. The bland appearance contrasted with marked perineural spread. There was no evidence of peritumoural invasion into lymphatic capillaries or blood vessels. Moderate amount of desmoplastic stroma and few groups of lymphocytes were present as well (Fig.2). By immunohistochemistry, S-100 protein was found in the tumour cell nuclei and cytoplasm (Fig.3). The neoplastic cells did not contain pan-cytokeratin, smooth muscle actin, desmin, estrogen and progesterone receptors, melanosome protein HMB-45 and p53 protein. The proliferation fraction by Ki-67 was low: 1%. The resection margin was free of tumour. The axillary lymph nodes were free of malignancy (0/7). Thus, the final diagnosis was granular cell tumour with wide invasive growth and perineural spread, without lymph node and resection line involvement. Twelve months later, the patient is free of recurrence.

## DISCUSSION

The history of granular cell tumour classically is associated with Abrikossoff who described granular cell tumour of the tongue in 1926. However, the first hints can be found in the works of Weber, 1854 (Brown *et al*., 2011). The GCT histogenesis is unclear. Abrikosoff proposed GCT origin from striated muscle cells and therefore named the tumour as myoblastoma. Subsequently, the immunophenotype of GCT with characteristic nuclear and cytoplasmic expression of S-100 protein and lack of myogenic markers (Filipovski *et al*., 2009) suggested neural origin from Schwann cell. This hypothesis is the most widely accepted nowadays. The granular consistency and large volume of the cytoplasm also is in accordance with the neural origin (Mittal and True, 1988). The GCT cells form intracellular myelin-like invaginations analogous to myelin layers surrounding nerve fibres. The infoldings become autophagocytosed by lysosomes resulting in granular consistency of the cytoplasm. The high number of lysosomes is indirectly confirmed by CD68 positivity.

The development of GCT in breast is known since 1931, when Abrikossoff described such occurrence only 5 years after his first publication of this tumour (Brown *et al*., 2011). Approximately 5-15% of GCT cases are located in the breast. GCT prevalence is estimated as 1:1000 in comparison with breast cancer. However, with the development of mammography the prevalence is growing and has reached 1:617 in the screened group and 6.7: 1000 in total population (Brown *et al*., 2011).

Thus, at present the problem of GCT is changing from extremely rare tumour to more practical, challenging differential diagnosis of breast cancer. Despite the success of screening, however, review of published cases suggests that until recently, most cases (70%) were found by palpation (Brown *et al.*, 2011) as in our patient. Clinically GCT can resemble cancer due to firm consistency, nipple and/or skin retraction (Lack *et al.*, 1980; Pergel *et al.*, 2011). These features can be attributed to stromal desmoplasia that was observed in our case as well. Less frequent manifestations include pain, multifocality, colocalisation with ductal carcinoma, tumour fixed to muscles, benign lymphadenopathy including reactive changes (Brown *et al.*, 2011). On mammography, GCT can show several features suggestive of malignancy: irregularity, spiculation, stellation, variable circumscription. However, calcifications are uncommon. US findings include solid heterogeneous poorly defined masses; these masses are hypo-echoic and display posterior shadowing. Thus, both US and MG frequently seems suspicious for malignancy. No radiologic data are specific for GCT. Fine needle aspiration and/or frozen section can be helpful but should be used with the usual caution regarding limits of interpretation. In contrast, the diagnosis in core or excisional biopsy is easy. It can be further justified by IHC. The immunophenotype in the presented case was characteristic. The association with nerve fibres also is in accordance with the described findings (Brown *et al.*, 2011).

The GCT treatment includes wide local excision. Lymph node excision or sentinel lymph node biopsy is not indicated except malignant cases or colocalisation with malignancy. Although conservative approach has been suggested (Patel *et al.*, 2008) it is not accepted. Extensive and/or combined treatment as for breast cancer should be avoided.

The prognosis is favourable. GCT can recur after incomplete resection but even in such cases the course remains benign. Few cases of malignant behaviour are documented (Brown *et al.*, 2011).

In conclusion, granular cell tumour can affect the breast and resemble cancer both clinically and radiologically. Due to benign biological potential and excellent prognosis, surgery should be limited to local excision. Misinterpretation as breast cancer and subsequent overtreatment should be avoided. Correct diagnosis can be reached by core biopsy and immunohistochemical investigation.

**Conflict of interest:** None

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## ACKNOWLEDGEMENT

This work was supported by ESF project Nr. 2009/0230/1DP/1.1.1.2.0/09/APIA/VIAA/070.

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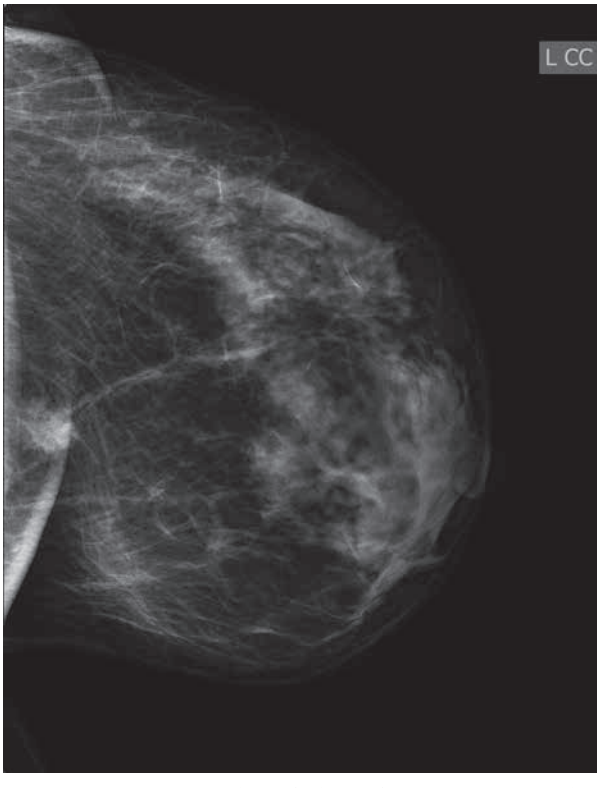


**Fig. 1. Mammography of the left breast. Note the stellate focus.**

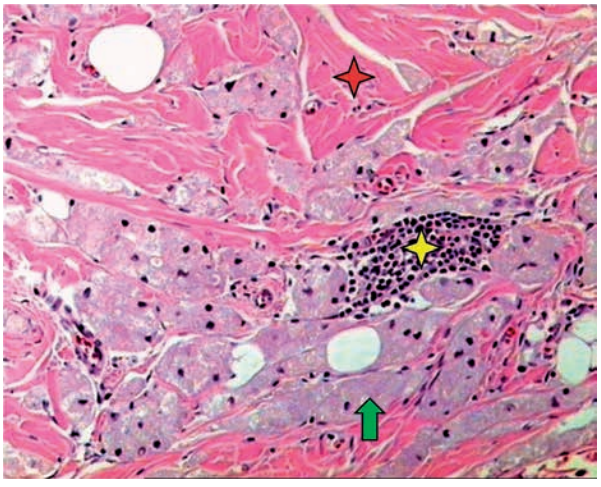

**Fig. 2. Microscopic structure of the neoplasm, typical for granular cell tumour. Note the large, bland tumour cells (arrow), desmoplastic stroma (red star) and small group of lymphocytes (yellow star). Haematoxylin-eosin, original magnification 100x.**

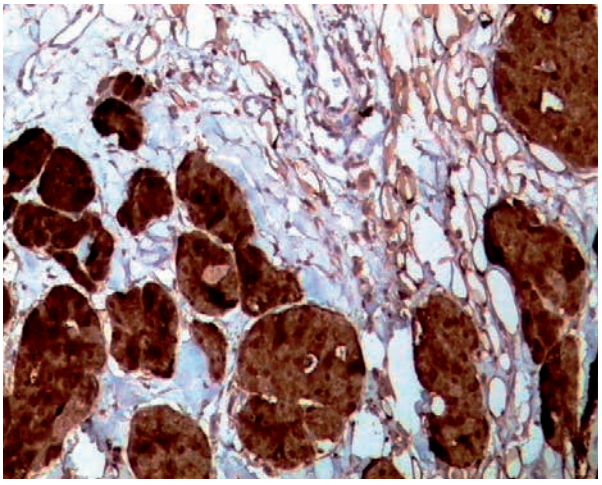

**Fig. 3. S-100 protein expression in the tumour cells. Immunoperoxidase, anti-S-100, original magnification 100x.**

10.2478/v10163-012-0017-5



*CASE REPORT*

# Superficial Temporal Artery – Middle Cerebral Artery Bypass in Moyamoya Disease Treatment

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**Summary**
Moyamoya disease is the chronic cerebrovascular disease, which is characterized by progressive occlusion of the intracranial part of internal carotid arteries and their proximal branches. Revascularization surgery of the ischemic hemisphere is a frequently recommended kind of treatment Moyamoya disease patients and it allows to prevent against stroke and to improve blood perfusion of the brain. We present the one case with treatment of this disease using superficial temporal artery (STA) – middle cerebral artery (MCA) bypass.
**Key words:** Moyamoya disease, STA-MCA bypass

## AIM OF THE DEMONSTRATION

The aim of this demonstration is to report about our experience in treatment of rare in our region patient with Moyamoya disease.

## CASE REPORT

55-year old woman was presented with history of ischemic strokes in the left anterior cerebral artery perfusion area 4 years ago and in the right middle cerebral artery perfusion area 2 years ago. Headaches, vomiting, dizziness, seizures, transient ischemic attacks, which are characterized as weakness or sensory loss during few minutes, speech difficulty, darkening of vision during few seconds, were presented in a current of 5 years and these neurological symptoms became more frequent and worse.

Preoperative MRI examination of the brain showed the old cerebral infarcts in the left frontal and right parietotemporal region.

Preoperative A right and left carotid angiograms (Fig.1) were performed with defuse stenosis of both side internal carotid arteries and the classical "puff of smoke" appearance was seen in the right and left cerebral circulation (Fig.2).


**Fig. 1 - Angiogram A, lateral projection, shows abnormal collateral vessels network (white arrows) and the angiogram B shows defuse stenosis of the internal carotid artery (black arrow) with occlusion of ophtalmica segment (white arrow).**

The first operation was done on the right hemisphere on 5th of January 2011 and the second – on the left hemisphere on 2nd of May 2011. In this surgical procedures were performed both side STA-MCA bypass, where STA was used as a donor artery and MCA as a recipient artery.

Few days after each operation patient felt much better and noted that arms and legs became agile and more powerful. During the year coming to examinations and controls patient had not complained of symptoms, which were presented before, except headaches.

Postoperative angiograms were performed, that, during the year, anastomoses work on both sides and were noticeably reduced quantity of Moyamoya vessels (Fig.3)

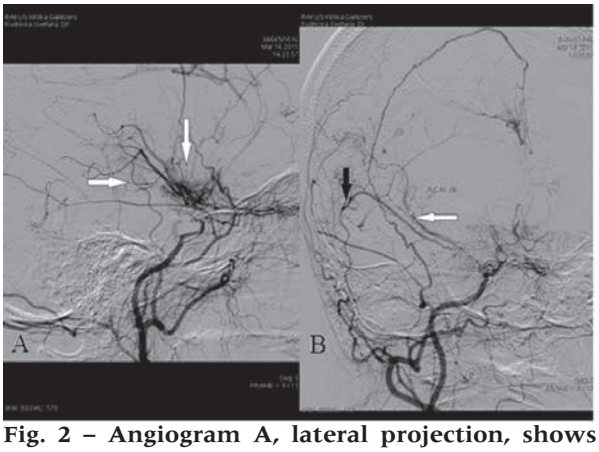


**Fig. 2 – Angiogram A, lateral projection, shows reduced quantity of abnormal collateral vessels (white arrows). Angiogram B shows excellent filling of the MCA (white arrow) from the STA-MCA bypass (black arrow).**

## DISCUSSION

The etiology of Moyamoya disease is unknown, but the pathological analysis had revealed, that this disease is characterized by the intimal thickening and attenuation of tunica media of the proximal vessels of the circle of Willis as well as the development of small collateral net-like vessels. The primary proteins that are currently implicated in the pathophysiology of Moyamoya disease include vascular endothelial growth factor, basic fibroblast growth factor, hepatocyte growth factor, transforming growth factor-$\beta_1$, and granulocyte colony-stimulating factor. Also the current literature has pointed to a low penetrance autosomal dominant or polygenic mode of transmittance at loci on chromosomes 3, 6, 8, 12, and 17 (13), as well as specific alleles of class II genes of the human leukocyte antigen have been described. (5)

Nowadays therapy is aimed on preventive actions against a stroke and to improve circulation of the brain, which helps prevent the appearance of symptoms and complications, which is characterized by this disease.

The surgery treatment is directed on creation of new blood supply for brain region with poor blood perfusion. There exist two main methods of surgical revascularization: direct and indirect methods.

Direct method includes the creation of anastomosis between branches of external carotid artery and internal carotid artery, and this method is typically used in treatment for adult patients. In general is used the superficial temporal artery to middle cerebral artery bypass. (4) This method the first time was performed by Yasargil in 1972 (2) and it allows immediately increase perfusion of the ischemic brain region.

Indirect techniques, which are traditionally used for children, involve the placement of vascularized tissues, which are supplied by the external carotid artery, in direct contact with the ischaemic hemisphere surface, leading to germinate new blood vessels to the underlying cerebral cortex. (12) There are few methods of indirect revascularization: the superficial temporal artery, which is left in continuity, or the temporal muscle on the ischaemic side of the head is placed in direct contact with the surface of the brain, as well as multiple burr holes procedure, in which several small holes are placed in the skull. Over time the result of angiogenesis is the formation of small arterial vessels, which germinate into the brain and increase the blood perfusion.(7) (8) (9) (10) There are possible negative effects of this method: necessary time for neovascularization, that means, that we cannot evaluate effect in short time after surgery, because of new vessels need at least few months to germinate into the brain and there is a possibility that process of angiogenesis could be not enough to provide normal perfusion of the brain.(3)

We decided to use STA-MCA bypass in treatment of our Moyamoya patient, because this is the most studied method of management Moyamoya disease, and this method allow immediately realise our main aim - increase blood flow in ischemic hemispheres. Also there is a possibility to use a combination of both revascularization methods with good short-term and long-term outcomes. This treatment could be used as a first step in Moyamoya disease treatment or after unsuccessful indirect surgery, when neovascularization process cannot provide necessary blood perfusion. (1) (4) (6) (11)

**Conflict of interest:** None

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