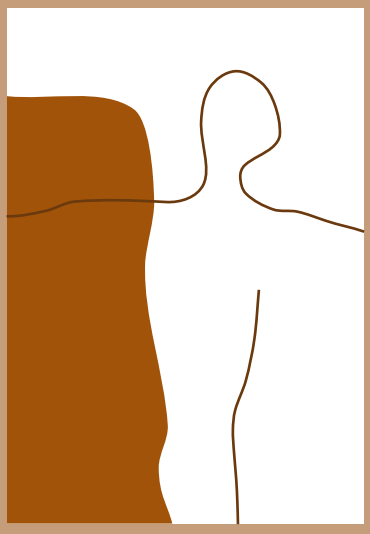

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ORIGINAL ARTICLE

Efficience of Cardiopulmonary Resuscitation in Emergency Medical Service and University Hospital

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Summary
Introduction. Survival rate among patients with cardiac arrest remains unclear. Return of spontaneous circulation (ROSC) is just the first step toward the goal of complete recovery from cardiac arrest. It depends on quality of cardiopulmonary resuscitation (CPR) during resuscitation and factors of postresuscitation care. Regular CPR analysis has not been carried out in Latvia.
Aim of the study was to evaluate survival rate among patients with out-of-hospital cardiac arrest and in-hospital cardiac arrest.
Materials and methods. The study was conducted in the State Emergency service of Latvia and Pauls Stradins Clinical University Hospital during 15 months in 2010/2011. There were 221 adult patients with in-hospital cardiac arrest and 162 adult patients with out-of-hospital cardiac arrest and performed CPR included in retrospective research. The information was analyzed by medical records. The obtained results were expressed in percents and compared, using the Pearson's Chi-square (Pearson $\chi 2$) test.
Results. The short-term ROSC was achieved among patients with out-of-hospital cardiac arrest (OHCA) in 62 cases and among patients with in-hospital cardiac arrest (IHCA) in 186 cases. Survival to discharge was achieved in 20.3% among patients with OHCA and 15.8% among patients with IHCA. The most commonly used CPR algorithm was pulseless electrical activity/asystole (72 - 73%). Short-term ROSC was achieved most frequently by ventricular fibrillation/pulseless ventricular tachycardia (41.3-56%), but the largest number of unsuccessful CPR episodes was observed by pulseless electrical activity/ asystole.
Conclusion. Results of CPR were different among patients with OHCA and IHCA. ROSC is rhythm-specific outcome.
Key words: in-hospital cardiac arrest; out-of-hospital cardiac arrest; return of spontaneous circulation; CPR.

INTRODUCTION

Cardiopulmonary resuscitation (CPR) is a complex of emergency procedures that, if performed correctly, can provide the necessary minimum of circulation until the return of spontaneous circulation (ROSC) is achieved (3; 4). ROSC is just the first step toward the goal of complete recovery from cardiac arrest. It depends on quality of CPR during acute resuscitation and factors of postresuscitation care. Survival rate among patients with cardiac arrest remains unclear. There is high heterogenity among CPR studies and they are not comparable (12). The European Resuscitation Council (ERC) Guidelines 2010 emphasize the quality of cardiopulmonary resuscitation and the post-resuscitation care as the big challenge for the next five years (5). Despite new techniques and technology it is not clear whether survival after cardiac arrest have improved (10). Therefore it is important to analyze the factors, determining the efficiency of the CPR such as patient's location at the moment of cardiac arrest: out-of-hospital or in-hospital (1; 11), the pathogenetic mechanism of the cardiac arrest: ventricular fibrillation/ pulseless ventricular tachycardia, pulseless electrical activity, asystole (2; 8; 9), implementation or improvement of training for medical persons, implementing of the resuscitation protocols in hospitals, the quality control of CPR during performing (4). The most common survival rate to hospital discharge range is reported being between 13.7% and 22.3% (10). It is worth mentioning that in the majority of cases it is possible to assess the quality of CPR only indirectly, according to the manikin studies in the process of training or according to the experimentally obtained data. The efficiency of CPR in decreasing the death-rate from avertable causes is a very important factor. In Latvia such systematic studies are not conducted at all (6). Therefore we should analyze the results and efficiency of CPR both out-of-hospital and in-hospital and look for ways to improve the situation in the future (3). The results of cardiopulmonary resuscitation during out-of-hospital cardiac arrest and in-hospital cardiac arrest were evaluated in that research – OHCA versus IHCA.

AIM OF THE STUDY

The aim of our study was to evaluate survival rate among patients with out-of-hospital and in-hospital cardiac arrest.

MATERIALS AND METHODS

The study was conducted in State Emergency Medical Service of the Republic of Latvia and in the Pauls Stradins Clinical University Hospital during 15 months in 2010/2011. Survival outcomes were evaluated using prehospital and hospital medical records. Cardiopulmonary resuscitation was performed according to the ERC Guidelines 2005 and 2010. There were 687 adult patients with a confirmed cardiac arrest, defined as unresponsiveness, apnea and the lack of

signs of circulation and performed CPR included in the retrospective research. 451 patient records with OHCA and 236 patient records with IHCA were analyzed. There were following factors evaluated: cardiac arrest location; medical team, who provided CPR; the pathogenetic mechanism of the cardiac arrest (ventricular fibrillation/ pulseless ventricular tachycardia, pulseless electrical activity, asystole) and survival outcomes. It was considered that CPR is effective if return of spontaneous circulation was established. Survival outcomes were categorized as short-term ROSC and long-term ROSC (7; 12). A short-term ROSC was defined as the return of spontaneous circulation for at least 20 minutes after the initial pulseless arrest and survival to hospital admission. A long-term ROSC was defined as hospital discharge. 304 patients were excluded from study – there were trauma patients, oncological patients, who received palliative care, and cases with missing data. Patients with OHCA were excluded from IHCA study. All CPR episodes were included for IHCA patients with multiple cardiac arrests (Table 2). The further analysis was conducted on 162 OHCA patients and 221 IHCA patients.

All OHCA occurred in presence of emergency medical service team. IHCA pacients were treated in general intensive care unit (ICU) and cardiological ICU with monitoring and medical staff on duty.

It was established that emergency medical service team and medical staff in hospital were similarly equipped with monitors, providing continuous registration of the vitally important parameters: the heart rhythm and heart frequency, the non-invasive arterial blood pressure and the level of oxygen saturation in blood. CPR was performed by trained prehospital personnel such as emergency physicians/reanimatologists, doctors assistants and trained ambulance car drivers and by hospital personnel such as physicians (emergency physicians/reanimatologists or cardiologists and trained nurses). An indication for admitting patients in the intensive care setting was haemodinamically unstable condition, a necessity for intensive treatment and the risk of cardiac arrest from reversible causes.

The impact of pathogenetic mechanism of cardiac arrest were analyzed in both groups, including ventricular fibrillation/ pulseless ventricular tachycardia – VF/VT, the pulseless electrical activity/ asystole - PEA/A and indeterminate rhythm.

Survival to hospital admission, survival to discharge and rhythm-specific survival was assessed in OHCA study. Short-term survival, survival to discharge and rhythm-specific survival were assessed in IHCA study. The obtained results were expressed in percents and compared, using the Pearson's Chi-square (Pearson $\chi 2$) test. The statistically valid p value was <0, 05.

RESULTS

Among included pacients mean age for male was 64 years, for female 70.5 years; most of patients were male (Table 1). The most frequent basic disease in IHCA study (Table 1) was of cardiac origin (n=101; 46%), followed by diseases of neurological origin (n=51; 23%). The baseline diagnosis in OHCA study was identified in 76.9% only, data were incomplete. Cardiac origin was assumed in 78% potencially identified OHCA diagnosis. There were 221 adult patients and 252 CPR episodes included in the IHCA study, the cardiopulmonary resuscitation on 31 patients was performed more than one episode.

The obtained data in IHCA study (Table 1) shoved that pulseless electrical activity/ asystole (PEA/A) was the initial heart rhythm in 73%, ventricular fibrillation/ pulseless ventricular tachycardia (VF/VT) in 23% and the initial heart rhythm was not determinate in 4%. ROSC was achieved most frequently in ventricular fibrillation/ pulseless ventricular tachycardia (Table 3), but unsuccessful CPR episodes were more common in pulseless electrical activities/ asystole. Using the parametric Pearson's chi-square (Pearson $\chi 2$) test, the value p was obtained, which indicated that the rhythm-specific differences of ROSC frequency were statistically valid (Chi2 $p<0.0001$).

OHCA data showed that initial heart rhythm was non-shockable (PEA/A) in 72.1 % and shockable rhythm(VF/ pulseless VT) was present in 27.9% (Table 1). The rhythm – specific short-term ROSC was achieved most frequently in cases of VF/pulseless VT (Table 3) and the difference was statistically valid ($p<001$).

Short-term ROSC was achieved in IHCA study (Table 2) frequently (n=186; 84, 2%), but the number of patients who were dischargexd from the hospitals after cardiac arrest was small (n=35; 15, 8%). 62 patients in OHCA study (Table 2) were admitted to Pauls Stradins University Hospital and Riga Eastern University Hospital after cardiac arrest and 33 patients (20.3%) were discharged alive.

DISCUSSION

CPR is one of the most important emergency actions in life-threatining conditions. Wallace et al. showed high heterogenity among studies related to the efficiency of CPR, suggesting that they are not comparable (12). The aim of this study was to assess the results and their determining factors of IHCA and OHCA, such as the efficiency of CPR depending on the patients location, and various pathogenetic mechanisms of the cardiac arrest. The demographic characteristics of patients age group corresponded with the general demographic trends. The average age between the genders did not differ much - it was 70.5 years for females, 64 years for males, however most of patients were male. The most common cause of cardiac arrest was on cardiac origin, which also corresponds with the statistic data of the Republic of Latvia.

Short-term ROSC was achieved in 186 episodes from 221 patients with IHCA, and 35 patients (15,8%) were discharged alive from the hospital. Short-term ROSC was achieved for 62 patients from 162 in OHCA group and 33 (20.3%) were discharged alive. These figures can be compared with the data published in separate studies. Girotra S.et al. shoved in a large, prospective, hospi-

tal – based, clinical registry of patients with in-hospital cardiac arrests in the United States that the overall rate of survival to discharge was 17% and there was a significant trend toward increased survival during 2000-2009 (10). The difference between IHCA and OHCA in our study should be evaluated with caution, because confounding factors were not identified and the possibility of residual confounding still remains. Unfortunately there was no accurate information on the neurological outcome in medical records at the time of discharge. Some studies showed that rates of severe neurological disability did not change significantly over time and improvement in survival rate has been accompanied by a decrease in the rate of neurologic disability among survivors (7). There were no detailed information on post cardiac arrest syndrome variables, like therapeutic hypothermia (10). Therapeutic hypothermia remains poorly implemented and further research is required, while the cognitive function is susceptible to many physiological and pharmacological perturbations during the acute periode (7). Very likely that better outcomes can be achieved with cooling but this hypothesis has been proven in clinical studies.

Analyzing the ROSC frequency depending on the pathogenetic mechanism of the cardiac arrest it is evident that the best results can be achieved if the first rhythm was the ventricular fibrillation or pulseless ventricular tachycardia, which corresponds with the data available in the literature. However Girotra S. et al. showed (10) that the proportion of cardiac arrests due to asystole or pulseless electrical activity increased during 2000-2009 from 68.7% in 2000 to 82.4% in 2009 ($p<0.001$ for trend)

The following problems were established during the study: 1) selection of primary outcomes varies in both studies; 2) improvements are necessary in documentation of resuscitation efforts, but same factors are often difficult to document accurately; 2) the neurological status were not evaluated. Future studies are needed to understand which factors are responsible for improvements in survival after cardiac arrest.

CONCLUSIONS

Results of CPR were different among patients with OHCA and IHCA. It should be interpreted in light of potential confounding factors and future studies are needed. We found that return of spontaneous circulation and survival rate are rhythm-specific outcomes. There is a statistically valid difference among ROSC frequence due to shockable and non-shockable rhythms. The ventricular fibrillation/pulseless ventricular tachycardia are associated with higher survival rate.

Conflict of interest: None

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Table 1. Baseline characteristics of patients with OHCA and IHCA

		OHCA	IHCA
Demographic characteristics			
Age (mean; years)	Female	73	68
	Male	63	65
Male sex (%)		58.5	52.0
Characteristics of cardiac arrest (%)			
VF		26.6	23
VT (pulseless)		1.3	
PEA		52.8	73
Asystole		19.3	
Not determinate rhythm			4
Baseline diagnosis (%)			
Cardiac origin		78.6	46
Neurological origin			23
Others		22.4	31

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Table 2. CPR results for patients with out-of-hospital cardiac arrest and in-hospital cardiac arrest

	OHCA	IHCA
Number of patients	162	221
Short-term ROSC	62 patients	186 CPR episodes
Long-term ROSC	33 patients (20.3%)	35 patients (15.8%)

Table 3. Rhythm – specific return of spontaneous circulation

	Short-term ROSC achieved (%)		**Short-term ROSC not achieved (%)**	
	IHCA	**OHCA**	**IHCA**	**OHCA**
VF/VT pulseless	**56**	**41.3**	**44**	**58.7**
PEA	**39**	**15.5**	**61**	**84.5**
Asystole		**14.9**		**85.1**

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ORIGINAL ARTICLE

MicroRNA Expression in Different Sybtypes of Breast Cancer

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Summary
Introduction. MicroRNAs are a class of small, non-coding RNA molecules able to regulate gene expression at the post-transcriptional level through binding to the 3'-UTR of the targeted mRNA, thus suppressing translation of the mRNA. In various diseases, including malignancies, expression of microRNAs is altered. Moreover, the altered expression of the microRNAs correlates with clinical and pathophysiological features of cancer thus making them good candidates for prognostic/predictive markers.
Aim of the study. The aim of this study was to determine expression level of five different microRNAs (miR-10b, miR-21, miR-29a, miR-31, and miR-214) in breast cancer tissues and to look for the differences in microRNA expression between distinct subtypes of breast cancer.
Material and methods. Forty five breast cancer and corresponding resection line tissues (control tissues) were studied. Breast cancer tissues were classified into the subtypes of triple-negative (23), luminal-A (13), luminal-B (7), and HER2+ (2).
Quantitative analysis of miR-10b, miR-21, miR-29a, miR-31, and miR-214 was performed by real-time PCR. The expression levels of microRNAs were normalized by the expression of the reference gene RNU6B.
The event-free survival in regard of high and low expression levels of microRNAs were analyzed by Log-rank (Mantel Cox) and Gehan-Breslow-Wilcoxon tests.
Results. Expression levels of four microRNAs (miR-21, miR-29a, miR-31, and miR-214) were significantly higher in cancer tissues than in corresponding resection line tissues. Breast cancer patients with low expression level of miR-21 showed a trend of better event-free survival than breast cancer patients with high expression level of miR-21; however, this trend did not reach statistical significance. In triple-negative tumor tissues, miR-21, miR-29a, and miR-31 showed significantly higher expression level than in luminal-A tumor tissues. Expression levels of miR-21 and miR-29a were significantly higher in triple-negative tumor tissues than in luminal-B tumor tissues.
Conclusions. Breast cancer patients with high expression level of miR-21 in tumor tissues show a trend of worse event-free survival, though; this trend did not reach statistical significance. Different microRNA expression in distinct subtypes of breast cancer points to the genetic heterogeneity of breast cancer, different regulatory targets and signaling pathways.
Key words: microRNA expression; different subtypes of breast cancer.

INTRODUCTION

Breast cancer is the most commonly diagnosed malignancy and foremost cause of cancer mortality among women worldwide. It is estimated that approximately 1.38 million new cases were diagnosed and 458,400 females died from breast cancer in 2008 (Jemal et al., 2011).

Breast cancer is clinically, morphologically and genetically heterogeneous disease and genetic changes between breast cancers differ, hence breast cancer treatment in the future must be based on patient's individual genetic changes using specific prognostic/predictive markers (Sjöblom et al., 2006; Stratton et al., 2009). Some of such prognostic/predictive markers are microRNAs. MicroRNAs (micro-ribonucleic acids) are a class of small, non-coding RNA molecules which regulate gene expression at the post-transcriptional level; through the binding to the 3'-UTR of the targeted mRNA, these molecules suppresses translation of the mRNA (Heneghan et al., 2010). In various diseases, including malignancies, expression of microRNAs is altered. In addition, altered expression of microRNAs correlates with clinical and pathophysiological features of cancer (Yan et al., 2008; Heneghan et al., 2010). One of the most studied microRNA which consistently is found up-regulated in wide variety of cancers, including breast cancer, is miR-21 (Huang et al., 2011; Mattie et al., 2006; Yan et al., 2008). Up-regulated expression of miR-21 in breast cancer tissues is associated with advanced clinical stage, lymph node positivity, and low survival rate (Huang et al., 2009; Yan et al., 2008). The expression of microRNAs varies not only within different types of cancer but as well as within different subtypes of cancer. The expression of some of the microRNAs (miR-21, miR-210, and miR-221) have been observed higher in the triple-negative (TN) subtype of breast cancer than in the corresponding healthy tissues; meanwhile the expression of miR-10b, miR-145, miR-205, and miR-122a has been observed lower (Radojicic et al., 2011). Some of the microRNAs have shown different expressions not only within the specific subtype of breast cancer but between distinct subtypes

of breast cancer as well. It has been observed that miR-210 is differently expressed between TN and estrogen-receptor positive/HER2 negative breast cancers: it was higher in the TN breast cancers than in the estrogen-receptor positive/HER2 negative breast cancers (Toyama et al., 2012).

AIM OF THE STUDY

The aim of this study was to determine the expression level of five different microRNAs (miR-10b, miR-21, miR-29a, miR-31, and miR-214) in breast cancer tissues and to look for the differences in microRNA expression between different subtypes of breast cancer.

MATERIAL AND METHODS

Forty five breast cancer patients hospitalized at Pauls Stradins Clinical University Hospital and/or Latvian Oncology Center from 2004 to 2011 were included in this study. All patients signed informed consent forms. Characteristics of breast cancer patients are described in Table 1.

According to the data of the immunohistochemistry, breast cancer tissues were classified into the subtypes of triple-negative (23), luminal-A (13), luminal-B (7), and HER2+ (2). Forty five corresponding resection line tissues were used as a control group. All breast cancer tissues contained more than 50% of cancer cells per sample.

MicroRNAs were extracted from the formalin-fixed and paraffin embedded tumor tissues and corresponding resection line tissues with the RecoverAll Total Nucleic Acid Isolation Kit (Ambion, Applied Biosystems). Reverse transcription was carried out with the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) on the TProfessional Thermal Cycler (Biometra). Quantitative analysis of microRNAs was performed by real-time PCR (Rotor-Gene 6000, Corbett) using TaqMan microRNA Assays (Applied Biosystems). Each sample was performed in three repeats. The expression levels were analyzed with the Rotor-Gene Q Series Software 1.7 using Comparative Quantitation Analysis. The expression levels of microRNAs were normalized by the expression of the reference gene RNU6B.

Event-free survival was calculated from the date of the diagnosis to the date of the first relapse, distant metastasis, or death from cancer, whichever occurred first. The event-free survival was analyzed using the Log-rank (Mantel-Cox) and Gehan-Breslow-Wilcoxon tests. Statistical significance was set at the 95% level ($p<0.05$). The median follow-up period of breast cancer patients was 33 months.

Wilcoxon test was used to assess statistically significant differences between tumor and corresponding resection line tissues. Whitney-Mann test was used to calculate statistically significant differences between TN and LA and TN and LB tumor tissues.

RESULTS

The expression levels of five different microRNAs between tumor and corresponding resection line tissues were compared. The median expression level ± SD (standard deviation) of miR-10b, miR-21, miR-29a, miR-31, and miR-214 in tumor tissues was 0.329 ± 0.462, 5.810 ± 6.932, 1.490 ± 2.403, 0.235 ± 0.609, and 1.000 ± 1.002, respectively. The median expression level ± SD of miR-10b, miR-21, miR-29a, miR-31, and miR-214 in corresponding resection line tissues was 0.198 ± 0.674, 0.726 ± 2.566, 0.618 ± 2.772, 0.044 ± 0.314, and 0.811 ± 0.475, respectively. The expression levels of miR-21, miR-29a, miR-31, and miR-214 were significantly higher in tumor tissues than in corresponding resection line tissues (Wilcoxon test; $p<0.0001$, $p=0.009$, $p=0.003$, and $p=0.002$, respectively). MiR-21 in tumor tissues was expressed most prominently compared to other microRNAs. Expression of miR-10b did not show any statistically significant differences between tumor and corresponding resection line tissues (Wilcoxon $p=0.095$) (Fig. 1.).

1. Event-free survival of 45 breast cancer patients were analyzed in relation with high and low expression levels of five microRNAs in tumor tissues. Expression levels above and below the median expression level of each of the microRNA were considered to be high and low expression levels, respectively. The median expression levels of each of the microRNA are described above. Breast cancer patients with high expression level of miR-21 was observed to have worse event-free survival compared to breast cancer patients with low expression level of miR-21, however, observation did not reach statistical significance (Log-rank (Mantel-Cox test); $p=0.13$; HR: 2.76 (95% CI: 0.73-10.45)). Statistically significant differences in relation with the event-free survival between high and low expression of miR-10b, miR-29a, miR-31, and miR-214 were not observed (Log-rank (Mantel-Cox test); $p=0.41$; HR: 1.75 (95% CI: 0.46-6.67), $p=0.99$; HR: 0.99 (95% CI: 0.26-3.73), $p=0.94$; HR: 1.05 (95% CI: 0.28-3.95), and $p=0.46$; HR: 0.61 (95% CI: 0.16-2.28), respectively (Fig. 2.).

2. The expression levels of miR-21, miR-29a, and miR-31 were significantly higher in the TN tumors than luminal-A tumors (Whitney–Mann test; $p=0.007$, $p=0.013$, and $p=0.002$, respectively). Expression levels of miR-10b and miR-214 did not show any statistically significant differences between TN and LA tumor tissues (Whitney–Mann test; $p=0.08$ and $p=0.234$, respectively) (Fig. 3).

The TN tumor tissues showed higher expression level of miR-21 and miR-29a than LB tumor tissues (Whitney–Mann test; $p=0.04$ and $p=0.035$, respectively). MiR-10b, miR-31, and miR-214 did not show any statistically significant differences between TN and LB tumor tissues (Whitney–Mann test; $p=0.105$, $p=0.063$, and $p=0.250$, respectively) (Fig. 4).

DISCUSSION

In this study, expression levels of miR-21, miR-29a, miR-31, and miR-214 were significantly higher in breast cancer tissues than corresponding resection line tissues. In numerous studies, expression of miR-21 has been found up-regulated (Mattie et al., 2006; Yan et al., 2008). MiR-21 is an oncogenic microRNA with anti-apoptotic potential which is directly involved in the growth, proliferation, and invasion of the tumor cells by inhibiting the activity of the tumor suppressor genes *PDCD4* (programmed cell death-4) and tumor suppressor tropomyosin-1 (TPM1) (Frankel et al., 2008; Zhu et al., 2008). The up-regulation of miR-21 in breast cancer has been associated with the advanced clinical stage, positive lymph node status, and overall patient's poor prognosis (Yan et al., 2008). Since the up-regulation of miR-21 is associated with the patient's poor prognosis, hence, the event-free survival of breast cancer patients in regard of high and low expression levels of miR-21 in tumor tissues were evaluated in this study. Although, statistical significance was not reached, a trend of worse event-free survival for breast cancer patients with high expression level of miR-21 was observed. Statistical insignificance could be due to the relatively small number of patients and relatively short median follow-up period (33 months).

MiR-31 is a tumor suppressor that prevents the progression of metastasis at the early stages of their development by inhibiting the activity of the metastasis-promoting genes: *FZD3*, *ITGA5*, *M-RIP*, *MMP16*, *RDX*, and *RXOA* (Valastyan et al., 2009). In non-metastatic breast cancer cell lines, miR-31 is up-regulated, while it is almost undetectable in metastatic breast cancer cell lines (Iorio et al., 2005). As in this study the majority of breast cancers patients at the time of diagnosis were non-metastatic, miR-31 was found up-regulated in the tumor tissues compared with the matched normal tissues. MiR-10b was the only microRNA which did not show statistically significant differences between the tumor tissues and matched normal tissues. MiR-10b is up-regulated in approximately 50% of metastatic breast cancers and down-regulated in non-metastatic breast cancers (Ma et al., 2007).

Some of the microRNAs in certain subtypes of breast cancer have been found expressed differentially. In the TN primary breast cancer tissues, compared with the matched normal tissues, expression of miR-21 has been found up-regulated (Radojicic et al., 2011). In our study each of the microRNA between different subtypes of breast cancer were compared. The expression levels of miR-21, miR-29a, and miR-31 were significantly higher in the TN than LA tumors; meanwhile, when compared with the LB tumors, only miR-21 and miR-29a were significantly higher. In another study, ERα-positive, ERBB2-negative, and PR-positive breast tumors showed significantly higher expression of miR-21 than ERα-negative, ERBB2-positive, and PR-negative breast tumors (Mattie et al., 2006). Expression of miR-21 is regulated by estradiol (E_2) in ERα breast cancers. When ERα-positive MCF-7 breast cancer cell lines were treated with E_2, the expression of miR-21 was suppressed (Wickramasinghe et al., 2009). Such findings explain why miR-21 is up-regulated in TN tumor tissues. Another microRNA which has been reported as being expressed differentially in the TN tumors is miR-31. In the TN primary breast cancer tissues expression of miR-31 was found down-regulated when compared with the matched normal tissues (Radojicic et al., 2011).

CONCLUSIONS

Breast cancer patients with high expression level of miR-21 in tumor tissues showed a non-significant trend of worse event-free survival. Although a trend did not reach a statistical significance, however this observation shows that miR-21 is a good candidate for prognostic marker in breast cancer.

Different microRNA expression in distinct subtypes of breast cancer points to the genetic heterogeneity of breast cancer, different regulatory targets and signaling pathways. The study will be continued.

Conflict of interest: None

Table 1. Characteristics of breast cancer patients

Characteristics	No. of patients (%)
Average age	55 (age range: 28–78)
Breast cancer subtype	
LA	13 (28.89)
LB	7 (15.56)
TN	23 (51.11)
HER2+	2 (4.44)
TNM stage	
I and II	31 (68.89)
III	13 (28.89)
No data	1 (2.22)
Lymph node status	
Positive	6 (13.33)
Negative	39 (86.67)
Relapse (Median follow-up period: 33 months)	
Yes	4 (8.89)
No	41 (91.11)
Metastases (Median follow-up period: 33 months)	
Yes	3 (6.67)
No	42 (93.33)
Death (Median follow-up period: 33 months)	
Yes	3 (6.67)
No	42 (93.33)

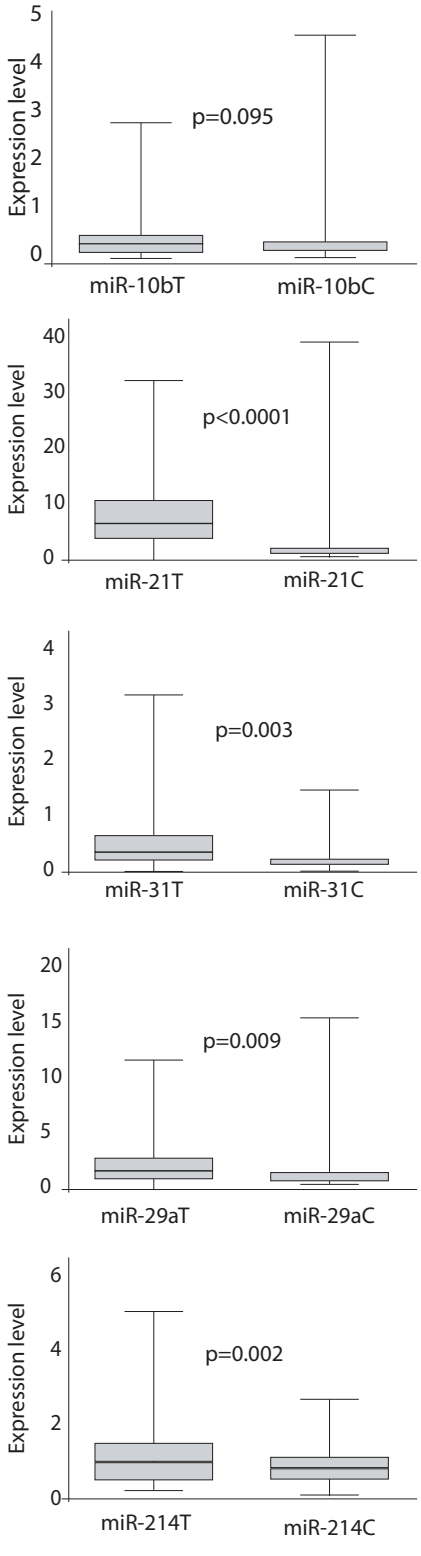


Fig. 1. Expression levels of five different microRNAs in tumor (T) and corresponding resection line tissues (C). Box-plot diagram with the median, first quartile, third quartile, and non-outlier range

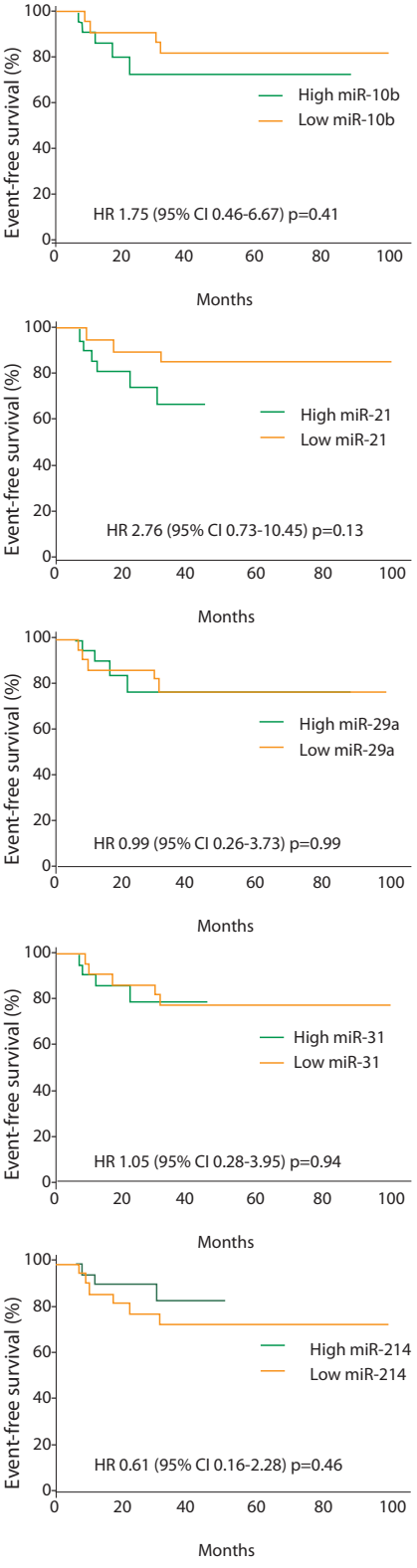
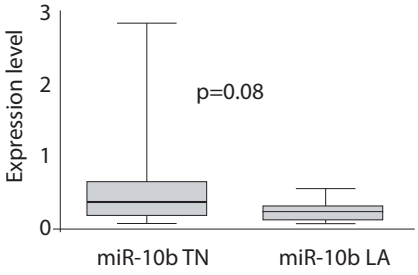
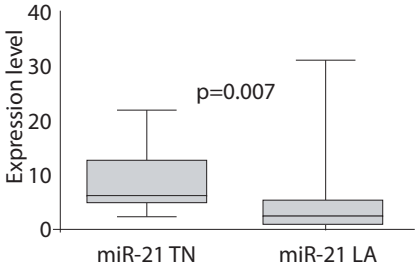
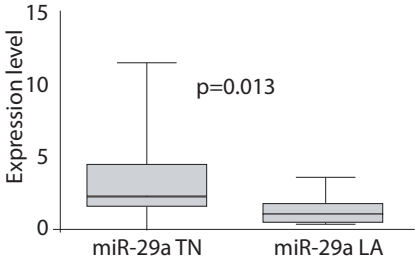
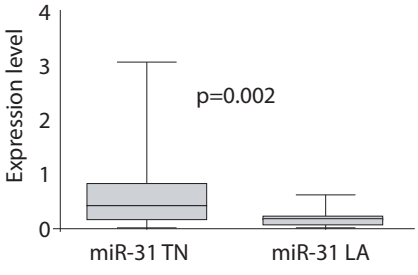


Fig. 2. Event-free survival curves of breast cancer patients with high and low expression levels of five different microRNAs. Statistical significance was set at the 95% level (p<0.05)









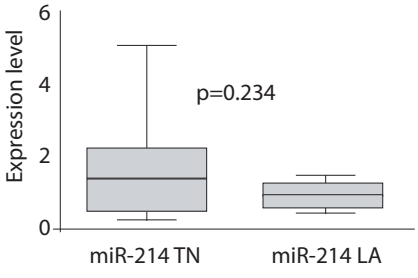
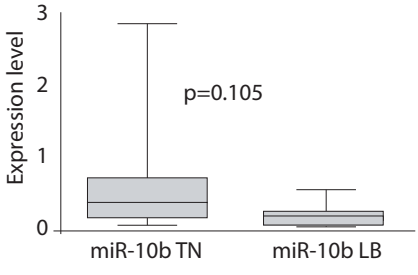
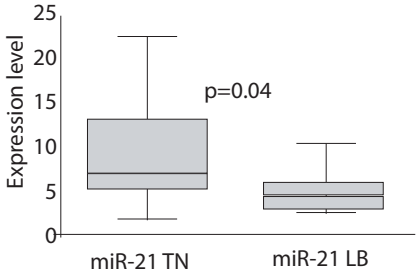
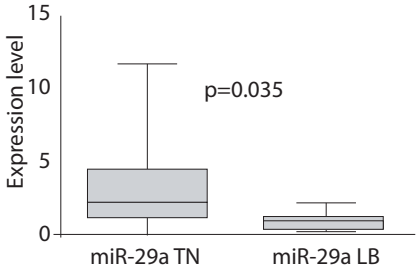
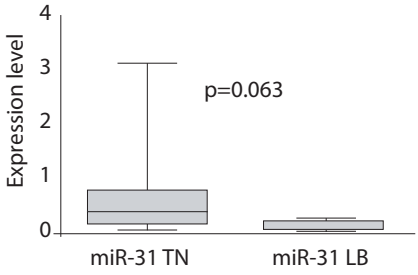


Fig. 3. Expression levels of five different microRNAs in triple negative (TN) and luminal-A (LA) breast cancer tissues. Box-plot diagram with the median, first quartile, third quartile, and non-outlier range









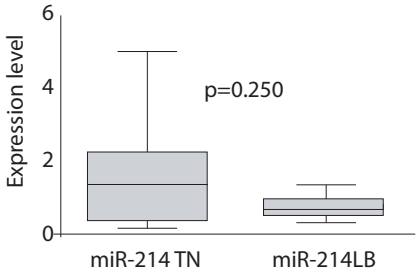


Fig. 4. Expression levels of five different microRNAs in triple negative (TN) and luminal-B (LB) breast cancer tissues. Box-plot diagram with the median, first quartile, third quartile, and non-outlier range

ACKNOWLEDGMENTS
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ORIGINAL ARTICLE

Application of Novel Methods for Non-Small Cell Lung Cancer (NSCLC) Biomarker Discovery

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Summary
Introduction. Research in NSCLC biomarker field and application of new methods is essential as it is promising strategy to reduce cancer mortality.
Aim of the study. The aim of the study was to develop new approach for lung cancer biomarker research and introduce novel method into surgical practice, verifying method's safety and evaluate intraoperative and postoperative complications.
Materials and methods. 50 patients with early stage NSCLC undergoing lobectomy were randomized into two groups – experimental (n = 25) and control group (n = 25). In experimental group at the time of thoracotomy and resection of the lung, paired blood samples were obtained from the lobar pulmonary vein draining lung segment containing tumor and from the peripheral vein which represents the systemic circulation. Safety of the procedure and its influence on surgery was evaluated.
Results. New approach for lung cancer biomarker research was developed. Injuries of major anatomical structures as pulmonary blood vessels, bronchi, trachea and pericardium due to blood sample collection were not observed. There was no difference in hospital stay and chest tube drainage duration. There were no perioperative deaths in both groups. Morbidity rate in experimental group was similar to control group.
Conclusions. Application of new method for developing multimodal lung cancer model is safe and does not increase the risk of intraoperative and postoperative complications.
Key words: biomarkers; lung cancer; lung resection.

INTRODUCTION

Lung cancer is the most common cancer worldwide with the number of incident cases around 1.6 million annually (14). Overall, survival rate at 5 years is less than 20% and the search for prognostic factors has led to extensive research and publication of an impressive number of papers. There are plenty of publications in the literature about lung cancer biological markers which can facilitate early detection of lung cancer in such a way improving results of treatment and improving 5-year survival. A blood based biomarkers are attractive targets because blood is easily accessible and measurements may be repeated over time (5, 9, 16). Blood is a medium which carries information about cellular processes, tumor progression and growth, signaling and much more (20). It is enormous amount of data which cannot be processed without building strategy allowing targeted approach. Isolating blood draining tumor gives insight into metabolism of cancer cells. Combination of surgical practice with new research strategies could help to achieve progress in NSCLC biomarker discovery. We describe a novel approach to biomarker discovery that used the same subject as control to identify elevated proteins in the pulmonary venous effluent draining the tumor vascular bed compared to systemic blood. This approach allows the differentlially present proteins to be identified against a complex and variable background of proteomic profile. The analytic issue is reduced to determining what has changed in an individual pre- and post- passage through the affected lung to get around the problem of finding specific biomarkers in blood.

AIM OF THE STUDY

The aim of the study was to develop new approach for lung cancer biomarker research, introduce and incorporate novel method into surgical practice, verify method's safety and evaluate intraoperative and postoperative complications.

MATERIAL AND METHODS

This study was designed as a randomized, case-control study. Development of novel approach was incorporated in a study design of Pauls Stradins Clinical University Hospital Department of Thoracic Surgery scientific project "Investigation of CXC group chemokines – novel diagnostic biomarkers for early stage lung cancer".
Patients with early stage (IA – IIB, 7th edition of TNM in Lung Cancer of the International Association for the Study of Lung Cancer) NSCLC undergoing lung resection with curative intent were involved in the study (n = 50) from January 2010 till December 2012. All patients participating in the study signed informed consent form.
A simple randomization was used. Patients were randomized into two groups – experimental (n = 25) and control group (n = 25). In all patients extent of resection was radical and restricted to lobectomy. Open lobectomy and nodal dissection was performed

in all patients. Steps of standard procedure were as follows - lateral thoracotomy, mobilization of lung, verification of tumor location, division of mediastinal and interlobar pleura, exposure and identification of hilar structures, dissection of pulmonary artery and vein, ligation and division of pulmonary vessels, dissection of lobar bronchus, division and closure of bronchus, haemostasis, drainage and wound closure. In experimental group at the time of thoracotomy and resection of the lung, paired blood samples were obtained from the lobar pulmonary vein draining lung segment containing tumor, and from the peripheral cubital vein which represents the systemic circulation. After dissection of pulmonary vein 5 mL of blood were aspirated with a syringe with 21-gauge needle prior to blood vessel ligation (Fig.1).

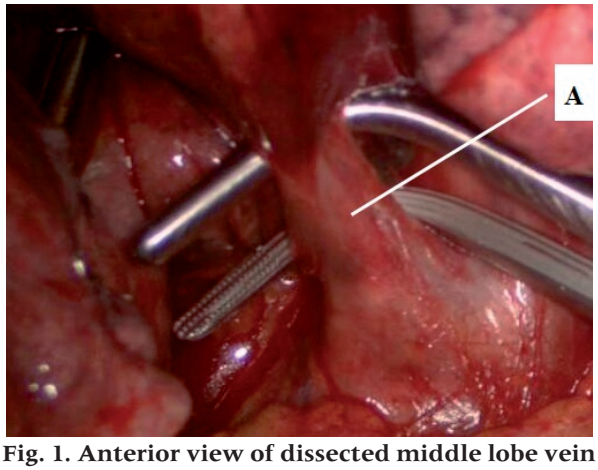

Fig. 1. Anterior view of dissected middle lobe vein of right lung before blood sample aspiration and vein ligation. Pulmonary vein puncture site (A)

Afterwards pulmonary vein was ligated proximally to puncture site. Blood samples were processed during next two hours after collection – centrifuged at 3000 g for 10 minutes and stored at -70°C for further analysis. Safety of procedure and its influence on surgery was evaluated. Various parameters were assessed including duration of surgery, intraoperative blood loss, postoperative hospital stay, duration of chest tube drainage, volume of postoperative fluid drained per day (postoperative day 1 to 3), haemoglobin concentration in blood and pleural fluid on the first postoperative day. Cardiac events were assessed intraoperatively and postoperatively – episodes of arrhythmia and miocardial ischemia were recorded. Data of two groups were compared.

Descriptive statistics and Student's paired t-test were used for the comparison of the means of numerical data.

RESULTS

Duration of surgery in experimental group was similar to control group (148.8 ± 37.1 minutes versus 141 ± 38 minutes, p = 0.47). Intraoperative blood loss was slightly higher in experimental group, but difference was not statistically significant (482 ± 177 ml (ranged 250 – 900 ml) versus 432 ± 146 (ranged 260 – 780 ml), p = 0.28). Volume of postoperative fluid drainage per day was similar in both groups with minor difference on the first day after surgery (Fig. 2).

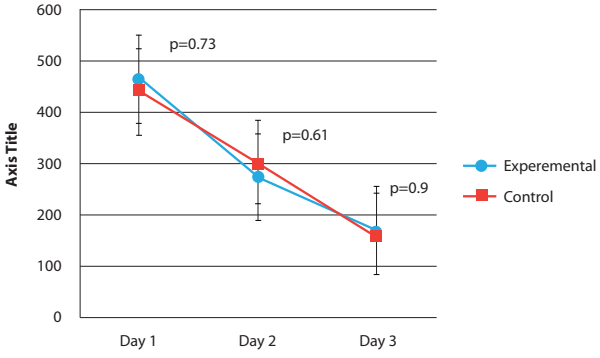

Fig. 2. Volume of postoperative fluid drainage per day (day 1 – 3). Values are expressed as the mean drainage (in milliliters) ± SE

Haemoglobin concentration in blood and pleural fluid on the first postoperative day was similar in experimental and control groups (Fig. 3).

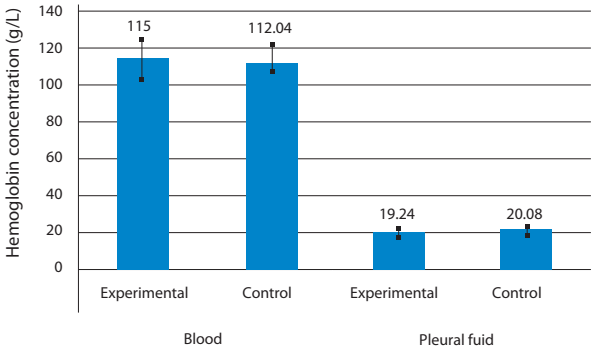

Fig. 3. Haemoglobin concentration in blood and pleural fluid on the first postoperative day. Values are expressed as the mean hemoglobin concentration (g/L) ± SE

Injuries of major anatomical structures as pulmonary vessels, bronchi, trachea and pericardium due to blood sample collection were not observed. Minor hemorrhage from pulmonary vein needle puncture site was observed in 5 patients (20 %). Duration of bleeding was restricted to several minutes (ranged 1 – 5 minutes) and related to the time necessary for pulmonary vein ligation.

There was no difference in hospital stay duration (7 ± 2 days in experimental group versus 8 ± 3 days in control group, p > 0.05). Chest tube drainage was longer in experimental group (6 ± 4 days) than in control group (5 ± 2), p = 0.04 (Fig. 4).

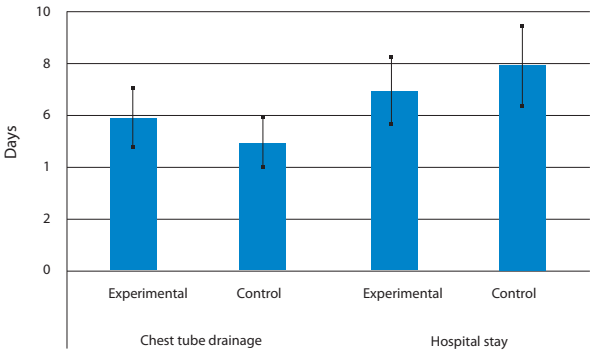

Fig. 4. Hospital stay and chest drainage duration. Values are expressed as the mean number of days ± SE

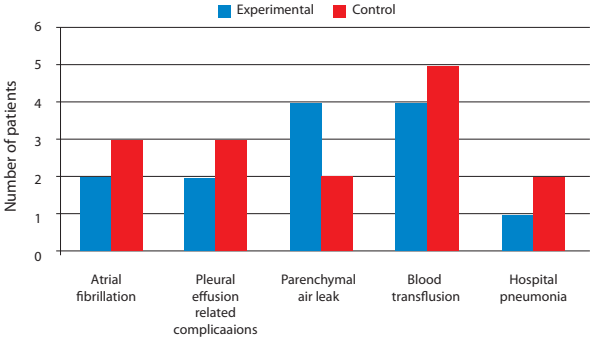

Fig. 5. Postoperative morbidity

There were no perioperative deaths. Cardiac complications were noted in one of the cases in experimental group (4 %) and three of the cases in control group (12 %) – atrial fibrillation developed and required treatment. In all cases arrhythmia successfully resolved during hospital stay. Four patients from experimental group (16 %) and five patients from control group (20 %) were given blood transfusion.

In two experimental cases (8 %) and three control cases (12 %) effusion-related complications developed that necessitated antibiotic treatment and prolonged drainage. Four patients from experimental group (16 %) and three patients from control group (12 %) had parenchymal air leaks persisting for more than 7 days. There were two cases of hospital pneumonia in control group (8 %) (Fig. 5.).

DISCUSSION

Research in biomarker field and discovery of novel potential biomarkers of NSCLC is essential and important as it is promising strategy to reduce lung cancer mortality. Single peripheral blood sample or *in vitro* tumor models not always adequately reflect complex metabolic processes and cellular interaction which can be found *in vivo*. Molecular theories based on experimental findings *in vitro* have limited impact on progress in cancer research (12, 15). On the other hand many clinical studies fail to isolate particular pathways in cancer cell biology due to complexity of molecular mechanisms, versatile function of receptors and ligands, as well as individual nature of every tumor based on unique genetical information. Up to date there is limited range of methods which make detailed analysis of cancer biology in vivo possible (2). As a rule with few exceptions these methods are invasive and ethical issues narrow spectrum of methods to be applied in human in clinical setting. Applied method allowed us to collect valuable material for further analysis with no impact on morbidity and minimal impact on course of surgical procedure.

Presence of pleural adhesions can sufficiently prolong the time of procedure, but what is more important – excessive tissue trauma during mobilization of lung can cause metabolic changes and affect potential lung cancer biomarker profile (3, 18). One of the main goals during surgery was careful handling of lung tissue, maximally short time interval between skin incision and collecting blood samples to minimize systemic response to general anesthesia and surgical trauma.

Variations of pleural fluid drainage can reflect extent of surgical trauma (4, 6, 11). Chest tube drainage duration was longer in experimental group, but further analysis revealed prevalence of lower lobectomies in experimental group. It is known, that due to anatomical features absorption of pleural fluid is more impaired in case of lower lobectomy than middle or upper lobectomy (8, 13).

Careful handling of pulmonary vessels during surgery is essential (10, 17), because minor tear in blood vessel wall can change course of operation or even affect extent of pulmonary resection. Major risk associated with novel approach is bleeding and injury of hilar structures which did not occur in any of 25 experimental cases. According to our results cardiac complications were not related to experimental procedure and occurred more often in control group, indicating possible relation to surgery itself.

One of main issues is use of analytic methods that do not provide precise and accurate determination of potential tumor specific proteins that are expressed in very low concentrations resulting in false negative results (1) – gaining access to tumor microcirculation and its unique environment holds promise to solve this problem.

CONCLUSIONS

Combination of surgical practice with new research strategies can help to achieve progress in NSCLC biomarker discovery. Pulmonary resection remains a procedure containing a high risk of postoperative complications by itself, but application of new method for developing multimodal lung cancer model is safe and does not increase the risk of intraoperative and postoperative complications.

Conflict of interest: None

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ORIGINAL ARTICLE

Chronic Total Coronary Artery Occlusion Recanalization with Percutaneous Coronary Intervention Using Anterograde and Retrograde Approach – Riga East Clinical University Hospital Experience

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Summary
Introduction. Despite advantages in interventional cardiology during last decade, chronic total occlusions (CTO) still remains one of the biggest problem in percutaneous coronary interventions (PCI) (10,14). CTO prevalence is high, but only less than 10% of percutaneous revascularizations are CTO interventions (17). There are no prospective randomized trials, properly powered for hard clinical endpoints, comparing modern optimal medical therapy with contemporary state of the art CTO recanalization (6).
Aim of the study. The aim of our study was to compare CTO PCI procedural parameters and treatment results using anterograde and/or retrograde approach.
Materials and methods. The study included all patients undergoing PCI for CTO at single tertiary PCI center between January 2007 and December 2012. 5568 PCI procedures were done in this period in our institution. 486 (8,64%) of them were CTO PCI. Retrograde approach was used in 138 (28,7% of all CTO PCI) cases. Patients were grouped according PCI year performing, approach (anterograde or retrograde) and PCI results (successful or unsuccessful). Demographic and procedural data were collected at the time of intervention.
Results. A total of 405 patients undergoing CTO PCI were included. The median age was 64yrs(38-88) and 79,2% was male. Retrograde approach (RA) was used in 138(28,7%) cases. RA usage has increase from 15.9% in 2007 till 46,8% of cases in 2012(p=0,0000218). The overall patient and procedure success rates were 77,8% (315/90) and 69,9%(340/146) respectively. Overall success rate has increase from 61,4% in 2007 till 87,1% in 2012 (p<0,001).
Overall survival was found better in patients group after successful procedure (Long-rank test p=0,019).
Conclusions. Retrograde approach usage significantly increase CTO PCI success rate, but doesn't increase risk of complications. Long-term outcome and survival after CTO PCI is not depending on approach (anterograde or retrograde), but on procedural success.
Key words: chronic total coronary artery occlusion; percutaneous coronary intervention; anterograde and retrograde approach.

INTRODUCTION

Despite advantages in interventional cardiology during last decade, chronic total occlusions (CTO) still remains one of the biggest problem in percutaneous coronary interventions (PCI) (10,14). According data from registries, CTO prevalence is high – CTO are encountered in 15% to 30% of patients undergoing coronary angiography (17). Compared with failed CTO PCI, successful CTO opening has been associated with angina relief, improvement of left ventricular function, avoidance of coronary artery bypass grafting and increased survival. Despite these benefits, less than 10% of percutaneous revascularizations are CTO interventions (17). CTO PCI is performed infrequently (4), likely due to historically low procedural success rates, technical complexity, high equipment use and the potential for major procedural complications (13).

PCI of CTO are also still relatively infrequent due to the uncertainty and paucity of data on the likelihood of successful PCI of CTO. There are no prospective randomized trials, properly powered for hard clinical endpoints, comparing contemporary optimal medical therapy with contemporary state of the art CTO recanalization (6).

The anterograde approach is the first technique chosen in the majority of PCI CTOs. However in many cases, antegrade wiring fails to cross CTO lesions.

In 1990, the first report of retrograde approach for CTO was published, in which the retrograde wire crossing technique was applied via a degenerated saphenous vein graft (SVG)(8). Later septal collaterals are considered to be potential access for retrograde approach. In 2005 Katoh and colleagues opened a new era of retrograde CTO recanalization with the Controlled Anterograde

and Retrograde sub intimal Tracking (CART) technique (15). Different kinds of retrograde techniques were introduced in the last years. Novel techniques hold promise in the field of percutaneous coronary intervention (PCI) for CTO (12).

AIM OF THE STUDY

The aim of our study was to compare CTO PCI procedural parameters and treatment results using anterograde and/or retrograde approach.

MATERIALS AND METHODS

Definition: A CTO lesion was defined as an obstruction of a coronary artery with Thrombolysis in Myocardial Infarction (TIMI) flow grade 0 with an estimated duration of at least 3 months. The duration of the occlusion was determined by the interval from the last episode of acute coronary syndrome consistent with the location of the occlusion or proven by previous angiography (2).

Multicenter CTO Registry in Japan has established as scoring system known as J-CTO score which can grade the difficulty in crossing a chronic total occlusion (CTO) of a native coronary lesion. The score was derived from the analysis of nearly five hundred native CTO lesions. J CTO score was defined as CTO complexity ratio, which is sum of anatomical findings and previous attempt results – predictors of procedure failure: blunt stump, CTO length>20mm, presence of severe calcification, proximal segment tortuosity and previous attempt failure (9).

The study included all patients undergoing PCI for CTO at single tertiary PCI center between January 2007 and December 2012. 5568 PCI procedures were done in this period in our institution. 486 (8,64 %) of them were CTO PCI. Retrograde approach was used in 138 (28,7% of all CTO PCI) cases. Patients were grouped according PCI year performing, approach (anterograde or retrograde) and PCI results (successful or unsuccessful). Demographic and procedural data were collected at the time of intervention (Table 1, 2). In-hospital MACE (myocardial infarction, urgent revascularization, stroke or death) was documented at discharge. Post discharge data was obtained by telephone follow up.

Descriptive analysis was used. Continuous variables were presented as mean ± standard deviation (SD). Categorical variables were presented as incidence (%). Statistical analysis was performed using SPSS 15.0 statistical software package.

RESULTS

A total of 405 patients undergoing PCI for 486 CTO were included. Number of patients and number of procedures was different, because 9 patients had 2 vessels CTO, but for 60 patients CTOs was attempted open twice or three times. The median age was 64yrs(38-88) and 79,2% was male. Retrograde approach (RA) was used in 138(28,7%) cases. RA usage has increase from 15.9% in 2007 till 46,8% of cases in 2012(p=0,0000218). (Figure 5) The overall patient and procedure success rates were 77,8% (315/90) and 69,9%(340/146) respectively. Overall success rate has increase from 61,4% in 2007 till 87,1% in 2012 (p<0,001) (Figure 4).

The target artery with CTO lesions included the left main (3 cases, 0,62%) right coronary artery (270 cases, 56,1%), the left anterior descending artery (165 cases, 34,3%), and the left circumflex artery (42 cases, 8,73%). Retrograde approach usage was for RCA 34%, LAD 23%, LCX 17% of cases.

We didn't find significant differences in patients clinical characteristics undergoing anterograde and retrograde procedures, except prior Coronary Artery Bypass Grafting (CABG). In retrograde approach patients group prior CABG had 15,2%, in anterograde group 6,4% patients. Mean CTO duration was longer in retrograde group - 50,4 month versus 35,4 month in anterograde group (Table 1).

Patients with diabetes (insulin dependent and non insulin dependent) was 22,45% in anterograde approach group and 17,5% in retrograde approach group (p=NS) (Table 1).

Three strategies for retrograde approach were applied: retrograde as primary strategy, retrograde immediately after antegrade failure and repeat procedure after previous antegrade failure. Retrograde approach as the primary strategy was applied in 29/137 (21,17%) patients, retrograde approach immediately after antegrade failure attempt was performed in 99/137 (72,26%) patients, and retrograde approach as elective procedure, after previously failed antegrade attempt, was performed in 9/137 (6,57%) patients. The success rate of these strategies was: 55% (20/23 patients) for primary, 61% (7/8 patients) for retrograde immediately after antegrade failure, and 44% (8/9 patients) for retrograde after previous failed antegrade attempt, respectively. Better success rate (61%) were founded in procedures group, whitch was done in one session – retrograde approach immediately after failed anterograde in comparison with 55% and 44% success rates in retrograde as primary and retrograde after previous failed anterograde respectively (Figure 6).

J CTO score was calculated for all patients. For less complex CTO lesions(J CTO score 0-1) retrograde approach was used in 5,1% and 19,8% of cases respectively, for intermediate complex lesions (J CTO score 2 and 3) in 28,5% and 42,3% respectively, for very complex lesions (J CTO score 4) in 70,6% of cases (p<0,001).

Septal collaterals were more frequently used as the retrograde access route (92.9%), but during last two years epicardial channels usage has increse.

The main reasons for failure in both groups (anterograde and retrograde) was inability to cross the occlusion with a wire (68,7% of all unsuccessful cases)(Table 2).

Total complications rate was 13,7%.In anterograde patients group 11,4%, in retrograde 19,6% (p=0,018). Main complications were coronary artery perforation – 6 cases in anterograde group (1,75% of all anterograde cases), 1 in retrograde (0,73% of all RA cases); intramural hemoatoma – 5 cases in anterograde group (1,46% of

all anterograde cases), 5 in retrograde (3,64% of all RA cases), access site complications -5 cases in anterograde group (1,46% of all anterograde cases), 3 in retrograde (2,19% of all RA cases)(Table 3).
In anterograde procedures group was found better survival results in successful procedures group, but difference was not significant (Long-rank test p=0,192). In retrograde procedures group also better survival was found in successful procedures group and difference was significant (Long-rank test p=0,012) (Figure 2).
In unsuccessful procedures group was found similar survival results in both – anterograde and retrograde groups (Long-rank test p=0,751).
Overall survival was found better in patients group after successful procedure (Long-rank test p=0,019) (Figure 1-3).

DISCUSSION

Revascularization of CTO, similar to stenotic vessels, is indicated in the presence of angina or ischemia related to the respective territory. The clinical presentation of a CTO can be very variable. On the one hand, there are patient with stable angina, silent ischemia or heart failure of ischemic origin. On the other hand for some patients CTO is incidental finding. Potential benefits of occluded artery opening are angina relief, left ventricular function improvement, coronary artery bypass avoiding (11).
Most of CTO patients are less symptomatic to compare with patients with stenotic lesions. Multiple registry experiences suggest that many patients with CTO do not receive either surgical bypass or percutaneous revascularization for the involved artery territory. Also multiple registers shows data suggest benefit of CTO opening (1, 7, 16). However not all CTOs benefit from revascularization. Main question is do we improve the prognosis with CTO revascularization. In Occluded Artery Trial (OAT) patients with a recent myocardial infarction of 3-28days, the interventional approach showed no advantage in terms of survival and more recurrent myocardial infarctions than in conservative approach. However this trial dealing with a different subset of patients, who had infarctions and only poor proof of viability or ischemia (5). In the Euro CTO register, the biggest ongoing CTO registry, only 18% of the patients presented with Q waves in the territory of the CTO vessel (11).
The main finding of this study is that the retrograde approach can be an effective tool for increasing the success rate of recanalization in complex CTO. Also study shows better survival after successful CTO opening. First successful CTO PCI using retrograde approach in Riga EAST hospital was done in December 2006. During last years operators have improve skills and retrograde approach usage in CTO cases have increased till 50%. Retrograde CTO cases was more complex, average J CTO score was 1,54 in anterograde and 2,45 in retrograde procedures group and better overall survival has been reached owing better results in retrograde group.
Retrograde approach is associated with potentially higher risk of complications. In our study we found higher complications rete in retrograde patients group, but difference was not statistically significant.

CONCLUSIONS

Retrograde approach usage significantly increase CTO PCI success rate. More complex technique (retrograde approach) usage doesn't significantly increase rate of complications. Indication of retrograde approach should be performed in case anterograde wiring seems very difficult in terms of anatomical factors. Long-term outcome and survival after CTO PCI is not depending on approach (anterograde or retrograde), but on procedural success.

Conflict of interest: None

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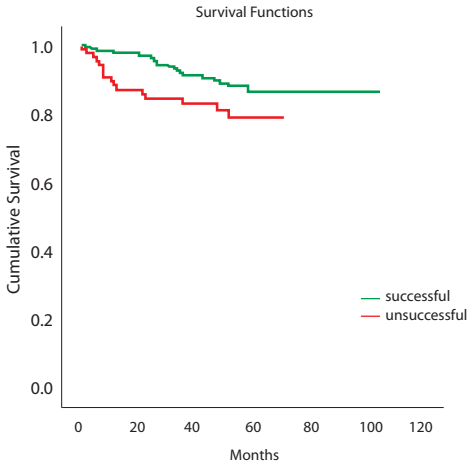

Fig. 1. Survival. Successful and unsuccessful cases

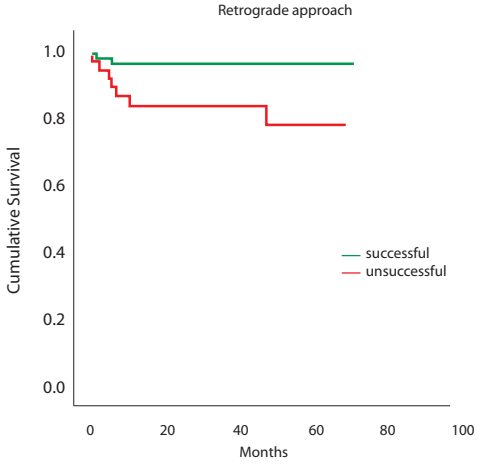

Fig. 2. Survival. Retrograde approach

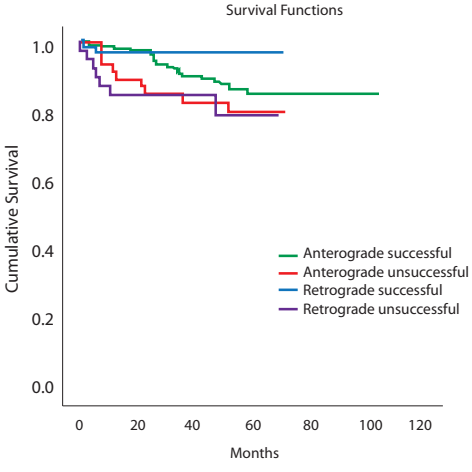

Fig. 3. Overall survival retrograde/ anterograde approach, successful/unsuccessful cases

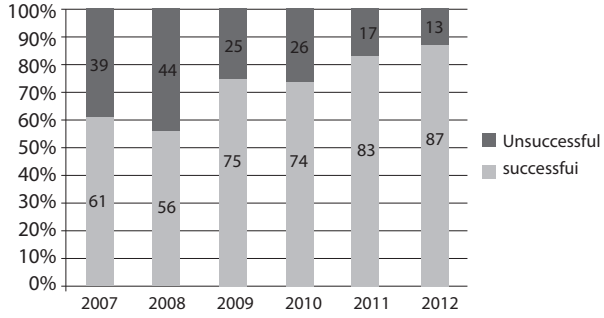

Fig. 4. Overall CTO PCI success rate in Riga EAST hospital

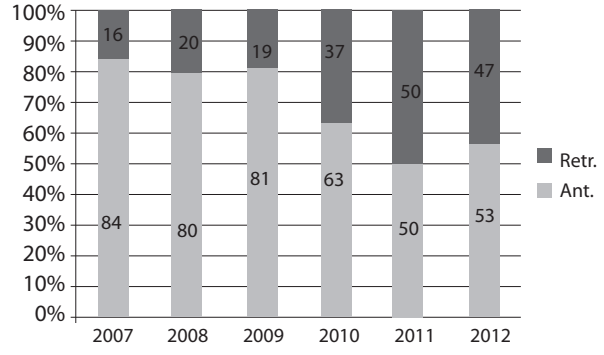

Fig. 5. Anterograde and retrograde approach usage in CTO cases

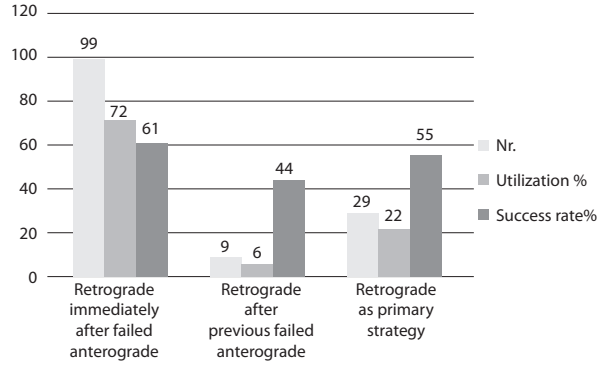

Fig. 6. CTO Retrograde cases strategy and success rates(n=137)

Table 1.CTO PCI patients baseline clinical characteristics (N=405)

Variable	Anterograde	Retrograde	p
Age	63,11(+/-10,28)	63,49(+/-9,99)	NS
Male	78,4%	81,15%	NS
Diabetes	22,45%	17,5%	NS
Smokers	32%	34%	NS
Hypertension	55%	61%	NS
Hyperlipidemia	47%	44%	NS
Prior MI	53,3%	50,0%	NS
Prior CABG	6,4%	15,2%	<0,001
Mean CTO duration (month)	35,4	50,4	<0,001

Table 2. Reasons for CTO procedure failure

	Anterograde failure N=89	Retrograde failure N=58
Wire was unable cross collaterals	-	14
Ballon delivery failure	8	4
Wire was unable to get through CTO body	75	25
Complications	6	3
Hight contrast volume, radiation explosure	0	2

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ACTA CHIRURGICA LATVIENSIS • 2013 (13)

ORIGINAL ARTICLE

Treatment of Abdominal Aortic Aneurysms with Accompanied Iliac Artery Aneurysms Using New Sack Sealing Device

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Summary

Introduction. 20-30% of abdominal aortic aneurysms (AAA) occur simultaneously with unilateral or bilateral common iliac artery aneurysms (CIAA). Endovascular aneurysm repair (EVAR) is known to be an effective AAA treatment method used by many centres in over 80% of cases. Presence of AAA accompanied by CIAA significantly increases the complexity of EVAR with currently available endografts, internal iliac artery (IIA) often requiring coil embolisation resulting in serious post-procedural complications such as ischaemia of pelvic organs, gluteal claudication and erectile dysfunction.

Aim of the study. Demonstrate successful endovascular AAA and CIAA treatment with new generation sac-sealing endograft device.

Materials and methods. From 2008 Pauls Stradins Clinical University Hospital is participating in the prospective clinical trial assessing the efficacy and stability of the new generation sac-sealing endograft device (Nellix®, Endologix, USA). Until now this trial had 40 enrolled patients with suitable for endovascular treatment aneurysmal morphology. The treatment group has included 16 patients with AAA extending to either one or both common iliac arteries (CIA). The control group consisted of 24 patients with isolated AAA. AAA diameter was 5.6±0.76 cm (min – 4.3, max – 6.98) and 5.16± 0.91 cm (min-3.78, max-7.24) in the treatment and control groups respectively. Seven patients had unilateral and nine patients had bilateral CIAA. The diameter of CIAA was 2.61±0.57 cm (min – 2.04, max – 4.44). Post-procedural follow-up was done at one, six and twelve months and on annual basis thereafter. During follow-up the general health condition of the patients was assessed as well as computer tomography angiography (CTA) and duplex ultrasonography (DUS) imaging was performed in order to examine the status of the aneurysm, endograft condition and patency of IIA. Statistical analysis of data was performed using v19.0 SPSS software (IBM).

Results. All patients successfully treated with new generation sac-sealing endograft excluding AAA and CIAA from blood circulation. Average follow-up period was 18 months. Upon follow-up in both groups endograft was stable and fixated in aneurysms with no endoleaks detected. In the treatment group all treated IIAs had remained patent with no pelvic organs ischaemia or gluteal claudication symptoms.

Conclusion. New generation sac-sealing endograft is effective and simple in employment for the treatment of concomitant AAA and CIAA allowing the treatment of aneurysms with complex morphology and preserving the blood flow to internal iliac arteries. Further studies are required for long-term assessment of this endograft efficacy.

Key words: internal iliac arteries; abdominal aortic aneurysm; endovascular aneurysm repair; aneurysm sac sealing device; Nellix device.

INTRODUCTION

Abdominal aortic aneurysm (AAA) is defined as an increase in aortic diameter larger than 3 cm and its prevalence in population aged between 59 to 79 years is 5.9% (18). In the event of untreated AAA the most common adverse event is aneurysmal rupture with mortality exceeding 75%. Even if the patient is admitted to hospital with ruptured AAA and treated by either open or endovascular repair, the associated perioperative mortality remains very high exceeding 50% (18). Therefore timely detection and elective AAA reconstruction is extremely important. Currently AAA reconstruction is possible using two methods: open and endovascular aneurysm repair (EVAR) (9, 18, 14, 23, 25). In the event of open repair AAA is resected and replaced with synthetic graft, whereas in the event of EVAR a self-expanding endograft is implanted into aorta under x-ray control with fixation being made in the relatively unchanged aortic segments proximally from AAA and distally in non-aneurysmal iliac arteries.

In 20 - 30% of cases AAA continues to either one or both common iliac arteries (CIA) with CIA diameter exceeding 20 mm (18, 27) (Figure 1). Currently available devices for AAA endovascular repair require unchanged iliac artery anatomy for distal fixation. If CIA is aneurysmatic then fixation is made in external iliac artery (EIA). In order to avoid retrograde filling of CIAA (type 2 endoleak) and prevent possible rupture, the internal iliac artery (IIA) must be occluded using coil embolisaiton or special occluders (2). Occlusion of IIA (and more importantly bilateral occlusion of IIAs) may result in important post-procedural complications

such as spinal cord and intestinal ischemia, impotence, gluteal claudication and necrosis of gluteal and/or perineal tissues (3, 6, 10, 21, 22, 24, 26, 28). The consequences of IIA occlusion depend on the blood supply requirements of pelvic organs that is usually more intensive in younger patients and patients with reduced cardiac output (7). The incidence of various complications after IIA embolisation during EVAR is provided in Table 1. The use of complex and expensive endovascular treatment technologies is necessary in order to keep blood flow to IIA (7). The development of new endovascular devices which would be simple in employment and allowing simultaneous treatment of both AAA and CIAA and preserving blood flow to IIA is therefore necessary. Available until recently endografts are based on exclusion of aneurysm from blood circulation and fixation in relatively healthy aortic tissues – that is in proximal and distal aneurysmal neck (14). However now there is a new generation sac-sealing endograft with fixation performed in aneurysmal sac. Special polytetrafluoroethylene bags are filled by rapidly freezing polymer and blood circulation continues through two endografts tubes 'frozen' in the aneurysmal sac (5, 12). To the best of our knowledge until now there have been no publications comparing such new generation sac-sealing endograft function in patients after combined AAA and CIAA treatment.

AIM OF THE STUDY

The aim of the study was demonstrate successful treatment of AAA accompanied by CIAA preserving the blood flow in IIA using new generation sac sealing endovascular device.

MATERIAL AND METHODS

Since 2008 the Vascular Surgery Department of Pauls Stradins Clinical University Hospital (PSCUS) in Riga, Latvia is the largest clinical centre participating in the multicentre clinical trial of the new generation sac-sealing endograft device (Nellix®, Endologic, USA) displayed in Figure 4. Ethical Committee of PSCUS approval was obtained for performance of this clinical trial and all patients have signed an informed consent form. Patients were enrolled into the study if the morphology of their AAA and CIAA allowed treatment with Nellix endograft, that is AAA > 4.5 cm or aneurysmal growth rate exceeding 10% in one year, AAA neck > 5 mm and diameter from 16 – 36 mm, patent femoral and iliac arteries allowing endovascular repair. Patients with ruptured AAA, thoraco-abdominal aneurysms, allergies to contrast medium, occluded iliac and/or common femoral arteries and patients with creatinine level exceeding 2.0 mg/dL were excluded from the trial. During this period 40 patients were successfully treated using Nellix endografts. Endovascular procedures were performed in the hybrid operating theatre equipped with angiography device (Siemens). Patients were divided into two groups. In the treatment group 16 patients had simultaneous AAA and either unilateral or bilateral CIAA. The control group consisted of 24 patients with isolated AAA. Demographic patient data is provided in Table 2. AAA diameter was 5.6 ± 0.76 cm (min – 4.3, max – 6.98) and 5.16 ± 0.91 cm (min-3.78, max-7.24) in the treatment and control groups respectively. In the treatment group 7 patients had unilateral and 9 patients had bilateral CIAAs. CIAA diameter was 2.61 ± 0.57 cm (min – 2.04, max – 4.44).

Patient follow-up was done at one, six and twelve months post-EVAR and on annual basis thereafter. General health condition, ankle-brachial index (ABI) and quality of life assessment was made during these post-procedural follow-up visits. The durability of Nellix endografts and blood flow in IIA was assessed using 64-layer CTA (General Electric LightSpeed) and DSU (Phillips iU22 xMatrix with multifrequence probe 2-4 MHz) performed by two experienced radiologists participating in the trial. Statistical analysis was done using SSPS software, v19.0 (IBM).

RESULTS

Long-term treatment results were prospectively analysed in patients after AAA and CIAA endovascular repair using new generation sac-sealing endograft device allowing successful CIAA treatment and preserving the blood flow to IIA. We assessed the efficacy and durability of the endograft, aneurysm related mortality and morbidity as well as post-procedural IIA patency.

All procedures were performed under general anaesthesia and all patients remained in intensive care unit for the period of one day after the procedure. All patients were successfully treated with implanted endograft excluding AAA from blood circulation. In the treatment group all patients' CIAA were successfully treated and in all cases blood flow was preserved to IIA (Figure 2). Iliac extenders were used for the treatment of CIAA in eight cases of the treatment group. There have been no peri-operative deaths, myocardial infarctions or pelvic organs ischaemia in either treatment or control group. The length of procedure in the treatment group was 134±32 min and 104±28 min in control group. T-test provided statistically significant difference in procedural length between two groups ($p<0.01$). Average blood loss in the treatment group was 85 ml (67.5-115) and 85 ml (65-125) in the control group. Mann Whitney U test has not provided statistical difference between two groups ($p>0.05$). The amount of polymer used in the treatment group and control group was 67.5 ml (57-92) and 48 ml (26.5-66) respectively. Average hospitalisation period was 7.5 days (4.5-10.5) and 5.5 days (4-6.5) in treatment and control groups respectively. Mann Whitney U test showed statistically reliable difference between hospitalisation period length and amount of used polymer between two groups ($p<0.05$). Length of procedure and amount of used polymer was larger in the treatment group due to the use of iliac extenders for CIAA treatment.

Average length of follow-up was 1.6 years (max – 4 years, min – 1 month). Neither of the patients have presented with clinical symptoms of pelvic organs' ischaemia, gluteal claudication or worsening in ABI. In all patients quality of life has returned back to pre-procedural level after one month post-treatment (11).

CTA and DUS follow-up in both group showed successfully excluded from blood circulation aneurysms (Figure 3). There have been no aneurysmal growth or endoleaks detected in either of the patients. All patients in both groups had patent IIA.

There have been two serious adverse events during the trial. In the treatment group on the 10th post-procedural month one patient with previously performed percutaneous transluminal coronary angioplasty (PTCA) died from myocardial infarction (MI). Another patient in the control group also with a history of PTCA has developed a MI with Q on the 15th post-procedural day and was treated with PTCA and stent implantation. Both adverse events have developed notwithstanding aspirin and statins therapy. This has not resulted in mortality and morbidity differences between two groups.

DISCUSSION

Endovascular repair of AAA and CIAA has proved its effectiveness in the last decade (9, 14, 23, 25) and is used as a method of choice in numerous European and North American centres. The greatest advantage of this method is a relatively smaller patient trauma, reduced peri-operative morbidity and mortality in the early follow up period. However endovascular methods are limited by complex aneurysmal morphology (short and angulated aneurysmal neck, iliac artery aneurysms and tortuosity) and combination of AAA with CIAA (19, 27). Similarly, the results of our study showed 40% of patients with AAA extending to CIA.

Currently there are no approved devices allowing simultaneous treatment of AAA and CIAA. Usually the treatment in such cases is performed with combination of different endovascular devices or employing complex unapproved endograft solutions during clinical trials. Devices used outside the approved indications increase risks of associated complications as well as lower treatment durability. The development of currently available devices increases their indications for employment in patients with complex AAA morphology (8). Specifically this is important for patients with CIAA where the preservation of IIA patency is more difficult. According to literature data IIA occlusion is associated with up to 80% of gluteal claudication, 10% of impotence and 6-9% of intestinal ischaemia cases (6, 27). In the event of pelvic organs ischaemia there is a significant increase in procedure related morbidity and mortality (15, 17).

Preservation of IIA in younger patients is especially important in order to retain quality of life. The analysis of 550 patients in *Farahmand et al* study has provided that the biggest risk for development of gluteal claudication was where IIA embolisation was used for occlusion. The risk of endoleaks however was not reduced in this group, regardless of occluded IIA (7). In our study neither of the patients with CIAA developed any complications.

One of the ways for preservation of IIA patency is combined endovascular and open repair where IIA is embolised during endovascular repair and thereafter reconstructed by open by-pass procedure from EIA or common femoral artery (CFA) to IIA (1). In this study performed by *Arco et al* two groups of patients were analysed first group received coil-embolisation of IIA before endovascular procedure and in other group IIA was covered with endograft and after patients was revascularised with open by-pass from EIA or CFA to IIA. Upon follow-up in the first group there were 21 patients with gluteal claudication and no complications observed in the revascularised group. The staged method applied in the second group somewhat contradicts the concept of non-invasive endovascular treatment where the procedure is performed through micro-incisions or completely percutaneously. This method also significantly increases the length and cost of the procedure.

Currently available alternative is endovascular treatment with branched endografts (13, 19). Also in this method there are limiting factors requiring CIAA diameter to be larger than 20-24 mm, without too tortuous IIA and EIA diameter of at least 6-8 mm with preferably no tortuosity. The requirement of such 'ideal' anatomy is necessary in order to implant a branched stent graft, however elderly patients and patients with large CIAA both IIA and EIA are usually significantly affected (10). As displayed by the latest studies with branched endografts it is possible to treat CIAA and preserve the blood flow to IIA, however this requires complex and expensive devices. This prolongs procedural time and decreases success rate of the procedure (16, 27). In addition to that, the increased complexity of the procedure reduces long-term outcome results. New generation sac-sealing endograft solution for the treatment of CIAA has provided that this device is not only effective but also simple in use and does not significantly increase the length of the procedure.

CONCLUSIONS

Our study shows successful treatment of AAA and simultaneous CIAA with new generation sac-sealing endograft. Eight cases required the use of iliac extenders to CIA, with the same simplicity in design and deployment, but only shorter in length. All patients had patent hypogastric artery on early and late follow-up. Serious adverse events during the trial in both treatment and control group were related to large prevalence of ischaemic heart disease in both groups. Further studies with larger number of patients and longer follow up period are necessary.

Conflict of interest: None

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Table 1. Incidence of complications after unilateral or bilateral IIA coil embolisation prior to EVAR

Author/year	Number of Patients	Gluteal Claudication	Spinal Cord Ischaemia	Erectile Dysfunction
Arko et al, 2004	12	6/12 (50%)	-	-
Bratby et al, 2008	39	12/39 (31%)	1/39 (2%)	2/37 (5%)
Engelke et al, 2002	16	4/16 (25%)	-	1/16 (6%)
Farahmand et al, 2008	76	44/76 (58%)	-	15/76 (21%)
Pavlidis et al, 2012	39	20/39 (51%)	-	-
Rayt et al, 2008	29	16/29 (55%)	-	-
Tefera et al, 2004	13	7/13 (53%)	-	-
Zander et al, 2007	14	4/14 (29%)	-	1/14 (7%)

Table 2. Demographic data in treatment and control groups

	Treatment Group	Control Group
Male	15	15
Female	1	9
Average age at the time of procedure (years)	70±8.20 (53-83)	70±6.99 (58-86)
Coronary artery disease	10 (63%)	8 (33%)
Peripheral artery disease	3 (19%)	5 (21%)
Hypertension	8 (50%)	12 (50%)
PTCA or CABG	6 (38%)	4 (17%)
Diabetus mielitus	1 (6%)	-
History of intra-abdominal surgery	4 (25%)	1 (4%)

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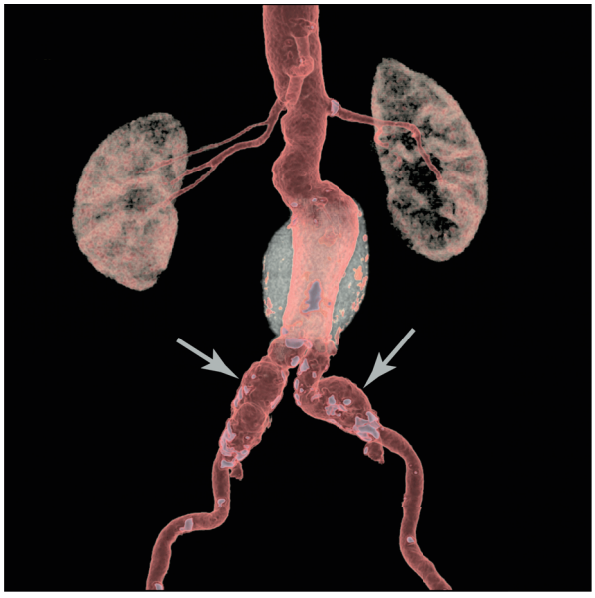

Fig. 1. CTA reconstruction in a patient with AAA combined with bilateral CIAA (arrows)

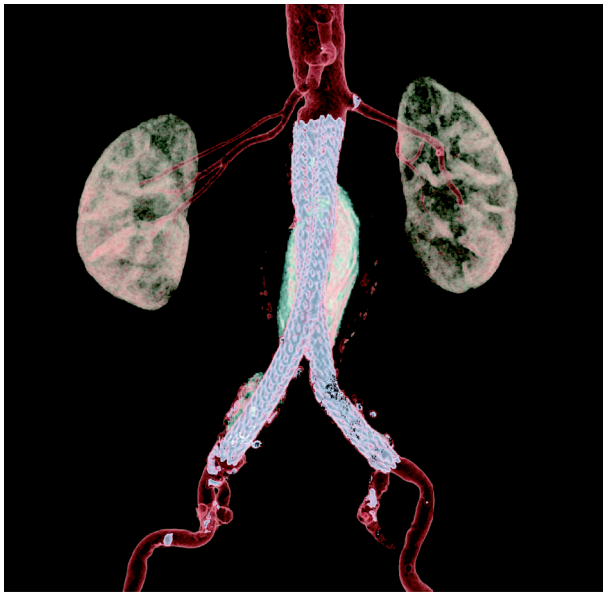

Fig. 3. CTA reconstruction in patient 3 years after procedure. Aneurysms excluded from circulation. Endograft is stable and fixed in the aneurysmal sac, blood flow preserved to IIAs

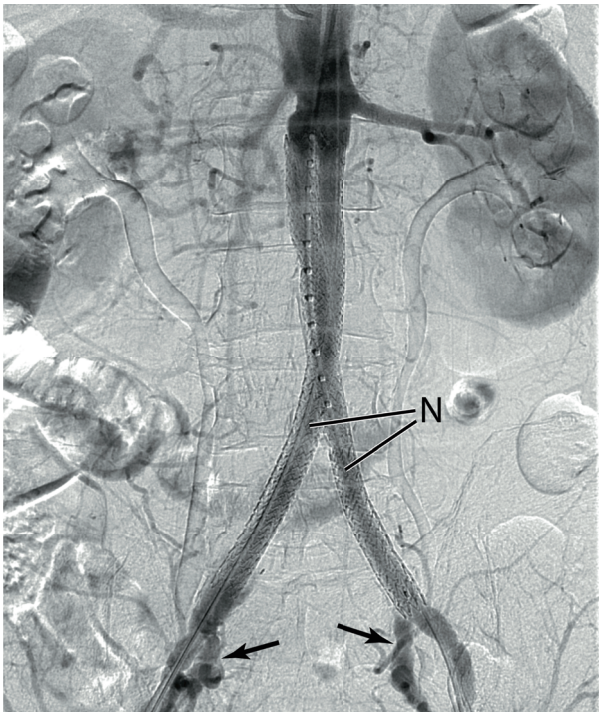

Fig. 2. Control DSA immediately after deployment of new generation sac-sealing endograft: AAA and both CIAA excluded from circulation, stent graft tubes frozen inside aneurysmal sac provide the blood flow continuance through endograft (N) to both patent IIA (arrows)

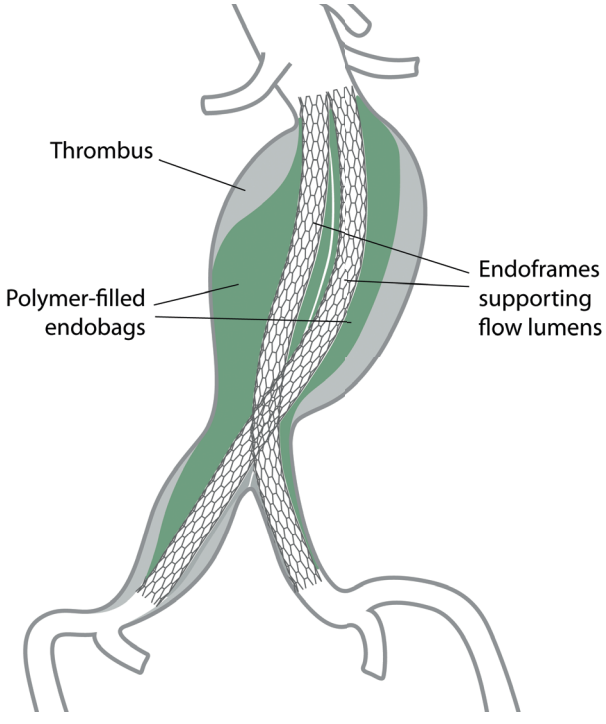

Fig. 4. Design of new generation sac-sealing endograft device (Nellix)

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ORIGINAL ARTICLE

Duplex Ultrasound Versus Computed Tomography For Follow Up Of Complications after Evar With Nellix Endograft: First Clinical Experience.

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Summary

Introduction. Contrast-enhanced computed tomography (CT) has become the 'gold-standard' imaging modality for surveillance following EVAR (2, 20). However repeated CT is related to increased cost, risk of contrast nephropathy and radiation exposure. Duplex ultrasound (DUS) is a less invasive but considered less accurate method than CT.

Aim of the study. The aim of this study was to determine the diagnostic accuracy of both imaging modalities for detection of complications in post-EVAR patients where the new generation sac-sealing endograft was used and to compare cost-effectiveness and sensitivity of both imaging modalities.

Methods. Analysis of 23 post-EVAR patients with implantation of new generation sac-sealing endograft device (Nellix®, Endologix, USA) was performed, making a comparison of CT and DUS. Contrast-enhanced computed tomography was taken as the 'gold-standard' investigation. DUS was compared to CT for analysis of sensitivity, post-imaging complications and cost-effectiveness. Statistical analysis of data was performed using v19.0 SPSS software (IBM).

Results. Analysis of CT and DUS studies compared in 23 patients. Both imaging modalities can detect AAA sac dimensions, endoleaks, and graft patency. The cost difference between two imaging techniques is substantial. Our results demonstrate that DUS surveillance during follow-up after EVAR where new generation sac-sealing endograft is used can accurately detect aneurysm size, endoleaks, graft deformations and stenotic or kinked graft limbs while lowering the overall costs of surveillance and eliminating CT related radiation and nephrotoxicity.

Conclusion. CT and DUS imaging can both detect AAA sac dimensions, endoleaks, and graft patency. The cost difference between the two imaging techniques is substantial. Our results demonstrate that in post-EVAR patients where new-generation sac-sealing endograft was deployed DUS surveillance performed by experienced radiologist can accurately detect aneurysm size, endoleaks, graft deformations and stenotic or kinked graft limbs while lowering the overall costs of surveillance and avoiding CT-related complications.

Key words: abdominal aortic aneurysm; endovascular aneurysm repair; aneurysm sac sealing device; duplex ultrasound; computed tomography.

INTRODUCTION

Endovascular aneurysm repair (EVAR) was first described in 1991 and is associated with a lower short- and mid-term morbidity and mortality (13, 14). However, such complications as endoleaks, endograft migration and deformations require life-long post-EVAR surveillance. The importance of these long-term risks is highlighted by recently presented data from the DREAM trial that shows greater 5-year post-discharge mortality in patients treated by EVAR compared with those undergoing open aneurysm repair (14). Endoleak in particular carries great significance, as it is predictive of post-EVAR rupture (27), and therefore, post-EVAR endoleak surveillance has become mandatory. At present contrast-enhanced spiral computed tomography (CT) angiography with specialized 3D reconstruction is considered as the gold standard for endoleak surveillance (1, 20). CT angiography is efficient in defining the anatomy of aneurysm sac, detection of endoleak and its classification but is associated with adverse factors including high dose of radiation, contrast nephrotoxicity and associated with contrast allergies, and high cost (5, 6, 23, 26). However upon development of new device technologies, and in particular introduction of new generation sac-sealing endograft device (12), DUS may be a good alternative to CT for the follow-up of EVAR patients. This modality is less expensive and does not carry the risks associated with ionizing radiation or contrast induced nephrotoxicity, however the sensitivity and specificity of DUS in comparison to CT in post-EVAR follow-up have been argued.,

AIM OF THE STUDY

The aim of this research paper is to update the sensitivity and specificity values of DUS in comparison to CT for patient follow-up after EVAR with new generation sac-sealing endograft.

MATERIALS AND METHODS

Nellix endograft is a new endoluminal sac-sealing device, which is designed to treat aortic aneurysms by obliterating the aneurysm sac, thus eliminating the potential endoleak space, while maintaining normal blood flow to the lower extremities. The endograft blood-flow lumens are supported with the balloon-expandable endoframes surrounded by the polymer-filled endobags, without the need for proximal and distal fixation. Full details of the device and clinical procedure are described in our previous reports (12, 17, 18).

23 post-EVAR (Nellix®, Endologix, USA) patients have been prospectively followed-up upon discharge, at six, twelve and twenty-four months at Pauls Stradins Clinical University Hospital (Riga, Latvia). The approval of ethical committee for the study was obtained and all patients have signed informed consent forms.

Two imaging modalities were used for post-procedural follow-up: DUS (Phillips iU22 xMatrix with multifrequence probe 2-4 MHz) with multifrequency probe (2-4, 12 MHz) and 64-layer CT (General Electric LightSpeed). DUS protocol included the assessment of AAA external diameter measurements in B-mode before and after EVAR in AP and transversal planes. Colour Doppler (spectral analysis, flow velocity) was used for stent graft, proximal neck and iliac arteries assessment. Contrast-enhanced computed tomography was taken as the 'gold-standard' investigation. Standard duplex ultrasound was compared to CT. Analysis was performed by two experienced radiologists participating in the trial. Statistical analysis was done using SSPS software, v19.0 (IBM).

RESULTS

All 23 post-EVAR patients have been prospectively followed up using DUS and CT imaging modalities. Four patients have been followed up for the period of six months, seven patients for the period of twelve months and twelve patients for the period of twenty-four months.

Measurements compared between CT and DUS are provided in Table 1. All separately analysed parameters are provided in Figures 1-7. AAA size correlation between DUS and CT in dynamical follow-up has provided good correlation between two imaging modalities ($r2=0.9379$, $r=0.9684$, $p<0.001$) (Figure 1) with DUS taking considerably shorter time of assessment (22±8 min, CT 94±28 min; $p<0.001$).

In one patient both DUS and CT detected type 2 endoleak on early follow-up. Another patient had a graft stenosis more than 50% detected by both DUS and CT, however DUS allowed more precise values by flow velocity determination.

DUS was found to be a considerably more cost-effective method (DUS 18.50 LVL and CT 146.00 LVL).

DISCUSSION

Although previous authors have compared DUS and CT scans for surveillance after EVAR, CT scan remains the 'gold standard' for assessment of aneurysmal diameter, detection of endoleak, and graft patency (2, 19, 20, 22). The benefits of CT as an imaging modality compared with DUS imaging include that it is highly reproducible, less influenced by body habitus, and offers faster image acquisition. However, among the limitations of CT are repeated radiation exposure, potential contrast-related complications, including allergy and renal insufficiency, and high costs (22, 23, 26).

AAA size reduction over time has been used as a surrogate marker for successful exclusion, thrombosis of the aneurysm sac, and decreased risk of rupture (23, 31). Many authors have shown that CT and DUS imaging are equivalent for measuring AAA sac size after EVAR (2, 19, 20).

Endoleak detection by DUS imaging in our study was as or more accurate than by CT, which is similar to the results provided by other authors (1, 27). Moreover, we believe that DUS imaging is more accurate than CT in detecting endograft related complications such as migration, deformation, kinking, and stenosis. Colour-flow images give physiologic as well as anatomic information that CT does not. We believe that DUS imaging can almost always accurately determine if structural defects are causing a flow-related problem and graft migration.

It was shown in previous studies that cost savings is substantial when DUS imaging alone is used for midterm and long-term follow-up versus the accepted approach that requires multiple CT scans (5, 6, 7, 22, 24). *Kim et al* estimated that current reimbursement for long-term EVAR surveillance and secondary procedures using traditional protocols average a net loss of $2235 per patient (16). Although hospital system charges vary by institution, in the setting of Latvian challenging economy the saving of 127.50 LVL (respectively 182.14 Euro). Inflation and decreasing reimbursements over time affect cost and charges, which makes a true cost analysis difficult. We performed our cost analysis using 2008 health care system charges to reflect the potential cost savings for the current economic climate and with today's health care system, which is significantly different than that of 1998, when our study began. Regardless, the cost savings are substantial when CT and DUS are compared for EVAR surveillance.

This study has some potential weaknesses. DUS imaging is more operator-dependent and has more interobserver variability than CT and is significantly affected by the patient's body habitus and fasting status. DUS imaging with contrast may prove to be especially useful for obese patients but is not necessarily any better in most patients, especially considering the extra cost and more difficult technique required to use this method.

The accuracy of DUS imaging to detect post-EVAR complications may vary depending on different graft designs, however, in our experience with new generation sac-sealing endograft we found that DUS is a better or at least as sensitive as CT in post-EVAR follow-up.

CONCLUSIONS

Although DUS is often used to augment CT scanning in post-EVAR follow-up, this evidence suggests that it is suitable for sole use in graft complications detection after EVAR. Our study confirms that DUS is a safe and sensitive modality for endoleak detection, graft migration and deformations detections, potentially obviating the need for patient exposure to high radiation doses and nephrotoxic agents in recurrent CT imaging. Further studies are required to understand whether DUS can completely replace CT imaging in the follow-up of patients after EVAR with new generation sac-sealing device.

Conflict of interest: None

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Table 1: Measurements compared between CTA and DUS

CTA	DUS
Transverse luminal size in the maximum stent graft deformation area	Maximal systolic blood flow (PSV) in the area of maximum stenosis
Luminal stenosis of stent graft in the maximum stent graft deformation area	Spectral blood flow in the external iliac artery
Angular deformation of the stent graft	

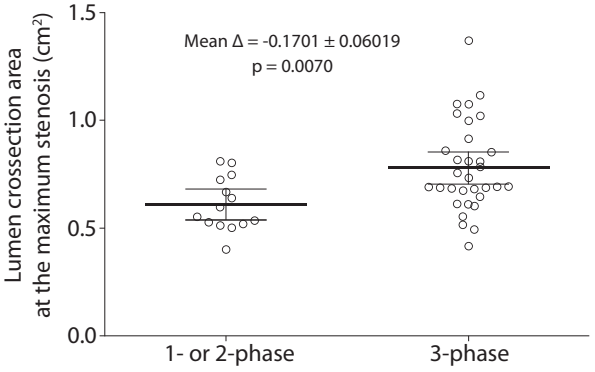


Fig. 1. The analysis showing that patients with 3-phased blood flow had a stent graft lumen approximately 17 mm^2 larger than those patients with a changed spectrum

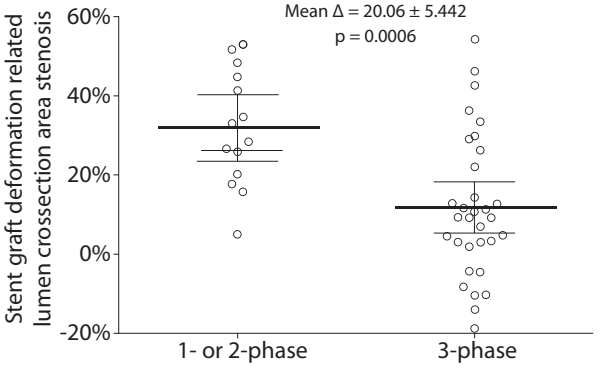


Fig. 2. Patients with unchanged blood flow spectrum had stenosis of stent graft most of the times < 20%. The average difference between registered stent graft stenosis in patients with changed and unchanged blood flow spectrum comprised 20%

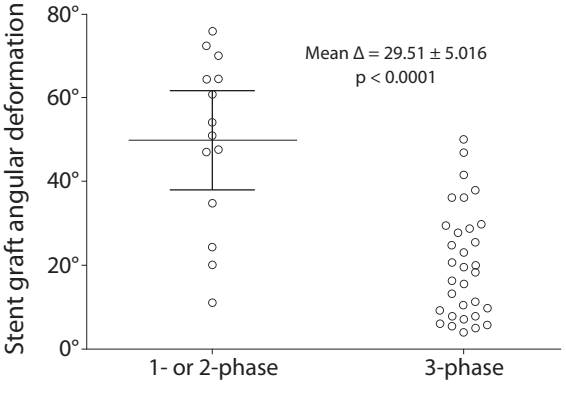


Fig. 3. Patients with changed blood flow spectrum had angluar deformation >30° than those patients with 3-phased blood flow spectrum

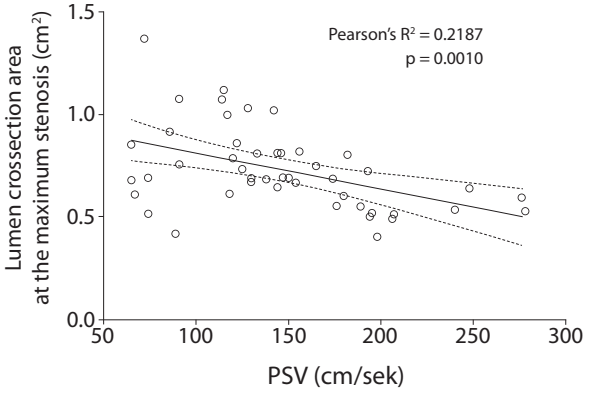


Fig. 4. Correlation between luminal cross-sectional area at the level of maximum stenosis and PSV is weakly expressed. Pearson's correlation coefficient is ~0.22, however this correlation is statistically significant (p=0.001)

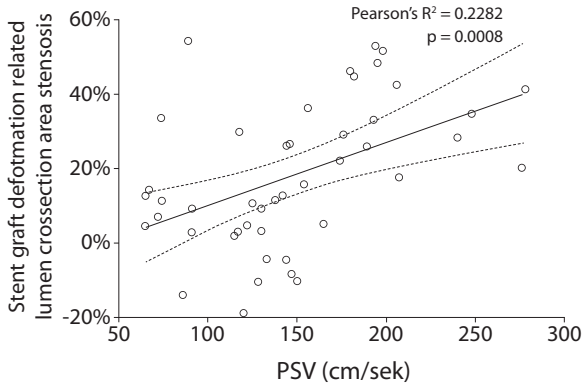


Fig. 5. Similar correlation was found also between cross-sectional stenosis and PSV

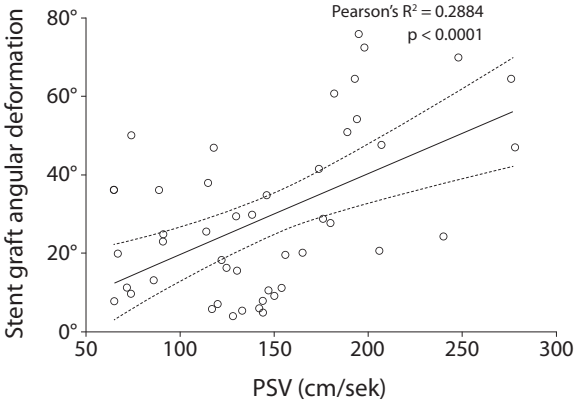
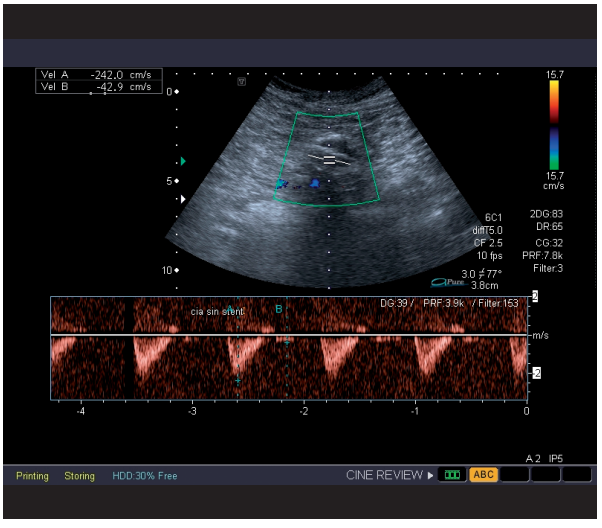


Fig. 6. Correlation of stent graft angular deformation with PSV approximatesd 0.3 with high statistical significance (p < 0.0001)



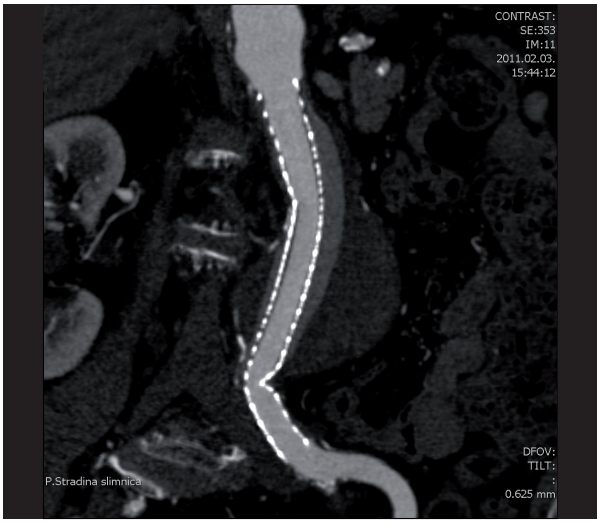


Fig. 7. Patient DUS and CT image in post-EVAR follow up with graft stenosis detected (242 cm/s in DUS equal to approximately 60% stenosis)

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ORIGINAL ARTICLE

Initial Findings of Breast Cancer Risk Factors from a Survey Conducted at Pauls Stradins Clinical University Hospital

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Summary
Introduction. Several risk factors for breast cancer have been documented based on epidemiological studies conducted over the last several decades - familial history of breast cancer, particular gene mutations, early menarche, late menopause, late age at first childbirth, use of oral contraceptives and hormone replacement therapy, absence of breastfeeding, alcohol use, smoking, obesity and physical inactivity
Aim of the study. Register data on breast cancer risk factors among the Latvian population and analyze it in relation to age of diagnosis.
Materials and methods. This paper presents quantitative survey data collected from April 2010 to June 2011 at the Pauls Stradins Clinical University hospital from 150 patients undergoing breast cancer treatment.
Results. A small correlation could be seen between age and patient menarche (0.1541; p=0.0749), as well as menopause onset (0.1286; p<0.0001). Hormonal replacement did not show any correlation with age, yet hormonal contraceptives had a moderate correlation with a statistical significance (–0.4988; p=0.0001). Lifestyle risk factors had higher correlations with age of diagnosis than reproductive factors. A moderate correlation could be seen for age and smoking (-0.37289; p<0.0001). A similar moderate correlation existed for age and use of alcohol (-0.31095; p=0.0012). Our survey found that no significant correlation exists between age of diagnosis and number of pregnancies, number of births and number of abortions. We found no significant correlation between age and length of breast feeding.
Conclusions. From current available data gathered in our study it can be concluded that for the Latvian population breast cancer risk is more associated with lifestyle factors than reproductive.
Key words: breast cancer; risk reproductive factors; lifestyle factors.

INTRODUCTION

Breast cancer is the most common form of cancer in women worldwide (16). Several risk factors for breast cancer have been documented based on epidemiological studies conducted over the last several decades. While it is difficult to identify a specific risk factor contributing to the development of breast cancer in a single patient, quantitative studies have identified the most likely factors to increase risk of developing breast cancer during lifetime to be familial history of breast cancer, particular gene mutations (*BRCA1, BRCA2* and *TP53*), early menarche, late menopause, late age at first childbirth, use of oral contraceptives and hormone replacement therapy, absence of breastfeeding, alcohol use, smoking, obesity and physical inactivity (5, 6, 2, 7). Besides genetic, reproductive and lifestyle factors, breast cancer risk varies greatly based on ethnic background and geographic locations, even within Europe it has been noted that highest rates of breast cancer are in the West and nearly twice lower in the East (5). Research analyzing differences in breast cancer incidence between developed and developing countries has shown the significance of diet, later childbirth, lower and shorter breastfeeding (11).

Very little research is available on the epidemiology of breast cancer risk factors in Latvia (6) with most research being done in the field of surgery and biomedicine. A potential source of information is the national register on patients with specific diseases where breast cancer data includes a notation of the patient having been linked to the following risk factors – family history of cancer, abortions, smoking and use of alcohol – but this data is limited for a multifaceted epidemiological analysis of breast cancer risk.

AIM OF THE STUDY

Register data on breast cancer risk factors among the Latvian population and analyze it in relation to age of diagnosis.

METHODS

This paper is based on a survey of breast cancer patients conducted from April 2010 until June 2011, during this time 150 surveys were collected.

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 the day after surgical treatment. 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.

Descriptive statistical data in this paper are presented as means, frequencies, proportions and percent of included patients. Reported confidence intervals were calculated at a 95% confidence level. Statistical significance was calculated using the Whitney Mann U test.

This study was approved by the Ethical Committee of Riga Stradins University and all patients involved signed informed consent forms.

RESULTS

The national cancer register in the year 2010 recorded 1057 breast cancer patients (13) 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 average age of patients was 62 (95% CI: 49-75) and ranged from 28 to 86 years. Approximately half of the patients were the age of 65 and older (48%, CI: 40-56). Nearly half of patients (48%, CI: 40-56) were married or living with a partner, 32% (CI: 24.5-39.5) were widowed, 13.3% (CI: 5.8-20.8) divorced and 6.7% (CI: 2.7-10.7) single. Majority of patients (47.3%, CI: 39.3-55.3) had a general secondary or vocational secondary education, more than one third (34.7%, CI: 27.1-42.3) had a university degree, while 18% (CI: 11.9-24.1) had primary and lower education, yet among patients older than 65 this was characteristic of 33% (CI: 25.5-40.5). Nearly half of patients (47%, CI: 39-55) were retired and living from state pensions, while 34% (CI: 26.4-41.6) were working, 5% (CI: 1.5-8.5) were housewives, 8% (CI: 3.7-12.3) were permanently disabled and only 4% (CI: 0.9-7.1) were unemployed.

Reproductive factors

As part of our questionnaire we gathered information on several reproductive factors: use of hormonal contraceptives and hormone replacement therapy; number of pregnancies, births and children; length of breastfeeding per child; age for start and end of menstruation; and number of spontaneous and induced abortions.

For most women menstruation ends around the age of 50, with the average age in the developed countries being 51.4 years (4), but distinct geographic and ethnic differences have been recorded around the world, for instance menopause comes slightly earlier in Southern Europe compared with Northern Europe (10). Our research found that the average age of menarche for patients was 14 (SD: ±2), with only 11% (95% CI: 6-17) of women having their menstruation start at the age of 12 or less. The average age of menopause was 50 (SD: ± 5), with only 15% (CI: 8-21) of women having their menopause at 55 or later (see Table 1).

Regarding use of hormonal medication we found that 21% (CI: 14-28) of patients had used hormonal contraceptives during their lifetime and 15% used hormonal replacement therapy. The average age of patients that had used hormonal contraceptives was 49 (SD: ±10) and for those that did not – 65 (SD: ±12) (see Table 1).

Average number of pregnancies was 3.34 (SD: ±2.14) with majority of patients having during their lifetime 1-5 pregnancies (77%, CI: 70-84). The average number of births was 1.72 (SD: ±0.96) with only 3% (CI: 3-6) having more than 3 births in their lifetime. In accordance there was an average of 1.65 (SD: ±1.73) abortions to a patient, with an average 1.40 (SD: ±1.58) induced and only 0.25 (SD: ±0.58) spontaneous abortions (see Table 1). Of all the abortions, 85% (CI: 82-89) were induced and 15% (CI: 11-19) were spontaneous.

Among the surveyed patients the average length of breast feeding during lifetime was 11 months (SD: ±9), with 62% (CI: 54-70) having breastfed only up to 12 months and 38% (CI: 30-46) longer (see Table 1).

Table 1. Reproductive risk factors

		%	Confidence Int.	Ave. Age	St. Dev.
Hormonal contracetives	Yes	21%	14% 28%	49	10
	No	79%	72% 86%	65	12
Hormonal replacement therapy	Yes	15%	9% 21%	60	10
	No	85%	79% 91%	62	14
Pregnancies	0	8%	4% 12%	63	15
	1	11%	6% 16%	59	16
	2	20%	14% 26%	60	14
	3	19%	12% 25%	63	13
	4	15%	10% 21%	62	13
	5	11%	6% 16%	61	13
	6	8%	4% 12%	61	12
	7	6%	2% 10%	67	11
	9	1%	-1% 3%	69	6
	12	1%	-1% 2%	61	-
Births	0	9%	5% 14%	60	17
	1	31%	24% 39%	64	12
	2	41%	33% 49%	60	13
	3	15%	9% 20%	65	14
	4	3%	0% 5%	53	8
	5	1%	-1% 2%	77	0

Breast feeding	0m	13%	8% 19%	60	16
	1-6m	25%	18% 32%	62	14
	6-12m	23%	17% 30%	60	12
	12-24m	25%	18% 32%	65	13
	≥24m	13%	7% 18%	61	13
Menarche	≤12	11%	6% 17%	57	13
	>12	89%	83% 94%	62	13
Menopause	<55	85%	79% 92%	62	13
	≥55	15%	8% 21%	55	11
Abortions	0	33%	26% 41%	60	14
	1	19%	12% 25%	63	15
	2	23%	17% 30%	62	13
	3	13%	8% 19%	62	12
	4	4%	1% 7%	60	10
	5	4%	1% 7%	66	14
	6	2%	-0% 4%	65	5
	7	1%	-1% 2%	73	-
	10	1%	-1% 2%	61	-

Table 2. Correlation between age and reproductive risk factors

	Correlation	P(1)	
Hormonal contracetives	-0.499	**Yes/No**	0.0001
Hormonal replacement therapy	-0.057	**Yes/No**	0.2177
Pregnancies	0.067	**<3/≥3**	0.3446
Births	-0.001	**0/≥1**	0.3372
Breast feeding	0.036	**<12/≥12**	0.1075
Abortions	0.080	**0/≥1**	0.1788

Lifestyle factors

As part of our survey we included also a variety of question related to lifestyle issues: duration and amount of smoking; pattern of alcohol use; weight and height; frequency of physical activity per week.

Among surveyed patients there were 25% (95% CI: 18-32) that had been smokers during their lifetime and 89% (CI: 84-94) had been alcohol users. Average age of those that had smoked was 53 (SD: ±12), while for those patients that had never smoked – 65 (SD: ±13). A distinct difference for age of diagnosis was also among patients that were not using alcohol on average 66 (SD: ±13) and for those that were using alcohol 60 (SD: ±13) (see Table 3).

The majority of breast cancer patients were overweight, with only 27% (CI: 20-34) being in the WHO normal body mass index range (BMI: 18.5-25), 37% (CI: 30-45) were overweight (BMI: 25-30) and 35% (CI: 27-43) were obese (BMI: ≥30). Average age of patients that were in the normal body mass index range was 58 (SD: ±14), while for those overweight – 63 (SD: ±12). The average height among patients was 1.63m (SD: 0.06) and the average weight was 75.63kg (SD: 16.36) (see Table 3).

Among the patients that answered about their weekly physical activities (110 patients), half (50%, CI: 42-58) had no physical activities or just once a week with an average age of 64 (SD: ±13), while the other half had physical activities 2 or more times a week (50%, CI: 42-58) with an average age of 59 (SD: ±13) (see Table 3).

Table 3. Lifestyle risk factors

		%	Confidence Int.		Ave. Age	St. Dev.
Smoking	Yes	25%	18%	32%	53	12
	No	75%	68%	82%	65	13
Use of alcohol	Yes	70%	63%	77%	60	13
	No	30%	23%	37%	66	13
Body Mass Index	Normal	27%	20%	34%	58	14
	Overweight	37%	30%	45%	64	14
	Obese I	22%	15%	29%	64	17
	Obese II	8%	4%	12%	63	7
	Obese III	5%	2%	9%	59	9
Physical activity	Non	17%	10%	24%	70	12
	Low	33%	24%	41%	61	12
	Moderate	29%	21%	38%	58	14
	High	21%	13%	29%	59	16

Table 4. Correlation between age and lifestyle risk factors

	Correlation	P(1)	
Smoking	-0.373	**Never/Had**	0.0001
Use of alcohol	-0.311	**Never/Had**	0.0012
Body Mass Index	0.192	**<25/≥25**	0.0069
Physical activity	-0.235	**≤Low/ ≥Mod.**	0.1788

DISCUSSION

A large epidemiological study done by Lacey et al. in 2009 showed that increasing age, nulliparity and use of menopausal hormone therapy were positively associated with breast cancer. Yet later age at menarche or menopause were less strongly associated with breast cancer than was expected. There were weak positive associations between severe obesity (8). A study done based on data from the Latvian national register on patients with specific diseases found that among breast cancer patients 10.6% had prior abortions, 3.7% smoked, 0.6% used alcohol (7). Our survey found that no significant correlation exists between age of diagnosis and number of pregnancies, number of births and number of abortions (see Table 2). In 2010 the total national birthrate was 34.4 per 1000 women aged 14-59. The national fertility rate was 1.18, while for breast cancer patients the average number of children was higher (1.72). The national abortion rate was 19.3 with a total of 10820 abortions in the year 2010, from all the abortions 68.7 % were induced and 12.1% were spontaneous (3). It is not possible to compare data on abortions for a single year and breast cancer patient lifetime abortions, but we can see that among the abortions in case of breast cancer patients there is a higher prevalence of induced abortions (85%). There are similar difficulties in comparing breast cancer patient data on number of births in a lifetime to the national statistics where we can see that on average the proportion of second child births among all births in 2009 was 34.11% and third child births – 11.31%, while among breast cancer patients 41% have had two births and 15% - three births.

An analyses of 47 international studies done by the Collaborative Group on Hormonal Factors in Breast Cancer showed that relative risk of breast cancer is reduced by 4.3% (95% CI: 2.9-5.8) for each year that a woman breastfeeds, in addition to a reducing by 7% (CI: 5-9) for each birth while being consistent for women from developed and developing countries of different ages, ethnic origins and various childbearing patterns. In this particular study the average age at diagnosis was 50 years, observed patients had fewer births than control groups, there was a greater proportion of nulliparous women and parous women who had never breastfed (1). We found no significant correlation between age and length of breast feeding, on average those having fed less than 12 months were diagnosed with breast cancer at the age of 61 (SD: ±13) and those that breastfed longer were an average age of 64 (SD: ±13). A weak correlation can be seen between age and patient menarche (0.1541; p=0.0749), as well as menopause onset (0.1286; p<0.0001). Yet while the hormonal replacement did not show any correlation with age at diagnosis, the hormonal contraceptives had correlation of –0.4988 with a statistical significance of p=0.0001 (see Table 2). The national data shows that 15.8% of all women aged 15-49 used hormonal contraceptives during 2009 (12), this proportion is lower than in our surveyed group (21%), but the national level only includes current users in a limited age group while we have surveyed lifetime use of hormonal contraceptives. Our survey of Latvian patients shows that lifestyle risk factors had higher correlations with age of diagnosis than reproductive factors. A moderate correlation of -0.37289 can be seen for age and smoking with a p<0.0001 where on average the age of patients that had never smoked was by 12 years greater. The national level of smoking for females in 2009 was 22% (17), while among breast cancer patients there were only 7% (CI: 3-11) current smokers, yet 25% had smoked during their lifetime. A similar moderate correlation was also for age and use of alcohol (-0.31095; p=0.0012), where patients that had never used alcohol in their life were on average diagnosed with cancer at 4 years later. Among breast cancer patients the proportion of lifetime abstainers was 11% (CI: 6-16), but among the Latvian female population 13.8% (data from 2003). Difference between national levels of current abstainers was smaller, yet still in total 31.7% did not use alcohol in the last 12 months while among breast cancer patients 30% (15).

A weak correlation (0.19212; p=0.0069) was noted for body mass index, with the average age of patients with a normal BMI being by 5 years less than those who were overweight. This can be attributed to the overall large proportion of older women, but considering women aged 45 and younger among the surveyed - 44% were overweight. Still compared to national BMI rates in 2008, among surveyed breast cancer patients there was a larger proportion of obese women (22% national; 35% surveyed) (18). Previous studies have shown that risk of breast cancer was lowest in lean women (BMI <22.8) who exercised at least four hours per week, but these effects were greater for premenopausal women (14). We found that in our survey for physical activity the correlation was small (-0.2350; p=0.015), with patients that had moderate or high physical activity a week being on average 5 years younger (see Table 4). This too can be explained with age specific patterns in physical activity, where the large cohort of older patients was also suffering from other health problems that limited their movement. The evaluation of body mass and physical activity should include a more detailed examination with adjustments to age and physical wellbeing, yet the current sample is too small for viable statistical analysis.

CONCLUSIONS

From current available data gathered in our study it can be concluded that for the Latvian population breast cancer risk is more associated with lifestyle factors than reproductive. The main impact on age of breast cancer diagnosis was seen from use of hormonal medication, alcohol and smoking. Some impact was also found for early menarche, late menopause, body mass and physical activity, but this data should be adjusted for age, other reproductive and lifestyle factors. To better evaluate several risk factor correlations a greater sample of data on breast cancer patients bust be gathered for further research.

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Conflict of interest: None

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ORIGINAL ARTICLE

Diagnosis and Treatment of the Primary Central Nervous System Lymphoma in the Riga Eastern Clinical University Hospital

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Summary
Introduction. Primary central nervous system lymphoma (PCNSL) is a rare tumor. It is diagnosed in 1-3% of all primary malignant tumors of the CNS. However its incidence increased over the past ten years amongst the immunesupressed and also the immunocompetent patients. PCNSL incidence amongst neurooncology patients is increasing in Latvia as well, therefore it is important to draw more attention to this problem.
Aim of the study. Research and analyse the experience of the Riga Eastern Clinical University hospital (RECUH) in the management of the patients with PCNSL over the last 11 years (from 2001 till 2012). In this research the data about the incidence of the disease, the connection between the age and sex, as well as the diagnostic and therapeutic possibilities are discussed.
Materials and methods. This is a retrospective descriptive study. In this study were included all the patients with morphologically confirmed PCNSL (n=18) that were operated in the neurosurgical clinic in the 11-year time period (from 2001 till 2012). The statistical analysis of the data was made by means of the Microsoft Excel 2010 and SPSS 20th version of the descriptive statistical methods.
Results. Over 11 years (from 2001 till 2012) PCNSL was confirmed in 18 patients, 17 (94.44%) of which were immunocompetent and in 1 patient (5.56%) HIV C1 stadium was diagnosed. Amongst immunocompetent patients 47.06% (n=8) were male and 52.94% (n=9) were female in the age between 45 and 79 years with average age of 64.41 years. Most often PCNSL was diagnosed in the age group between 65 and 69 years. 83.33% of all the PCNSL cases were diagnosed beginning with 2007. In all of the PCNSL patients a B-cell-lymphoma was morphologically confirmed. By admission the average Karnofsky Performance Scale Index was 57.78 in all patients, but after receiving a combination of therapy it was 77.78. The median survival amongst all of the patients was 515 days or 17 month, but amongst the patients, that received the full range combination therapy, the median survival achieved 867 days or 29 month.
Conclusions. The analysed data demonstrates that the incidence of PCNSL has mighty increased over the past six years and PCNSL is more often diagnosed in elderly, in which the KPS index and the median survival considerably increases after the combination therapy.
Key words: the primary central nervous system lymphoma; diagnosis; teatment.

INTRODUCTION

The primary central nervous system lymphoma (PCNSL) is classified as extranodular lymphoma, that is formed in craniospinal axis without having a systemic spreading. It generally localizes in the parenchyma of the brain, it can present itself in the eyes, leptomeninges and the spinal cord (5).

PCNSL is a rare tumor affecting the central nervous system (CNS). It is diagnosed in 1-3% of all malignant CNS tumors (10). The incidence of PCNSL amongst immunocompetent patients is about 0.28 of 100 000 people per year, but amongst AIDS patients the index equals 4.7 of 100 000 people per year (7). In spite of the small amount of patients its incidence has been increasing amongst immunosupressed and also immunocompetent patients over the past ten years (18).

PCNSL can affect patients at any age (21), most incidence is in the age range from 50 to 70 years, amongst immunocompetent patients and with mean age of 60 years (24,33). The age of the manifestatation amongst immunocompromised patients is less, for example, in patients with acquired immunodeficiency it is 10 years, after an organ transplant – 37 years and in AIDS patient – 39 years (21). The sex difference amongst immunocompetent patients is 3:2 (21), but amongst immunosupressed patients 95% are men (35). PCNSL is very rare in children and usually it associates with congenital immundeficiency, for example IgA deficiency, hyperimmunoglobulin M syndrome or Wiskott-Aldrich syndrome (14).

Up to now the origin of the malignant lymphocytes in PCNSL is not known, because there are no lymph-nodes or lymphatic tissues in the CNS. It has been proved that T-lymphocytes can cross the hematoencephalic barrier, but B-lymphocytes usually cannot be found in the structures of the CNS, even though the majority of the PCNSL cells has B-lymphocyte origin (27). Epstein–Barr virus (EBV) plays a role in the development of the PCNSL in immunosupressed patients. The genome of

the EBV can be found in 95% of the PCNSL cells and in 20% of immunocompetent patients (38). That could explain that the affected B-lymphocytes proliferate in the structures of the CNS and develop a tumor without the control of the immune system. However an assumed etiology of PCNSL hasn't been found in the immunocompetent patients (28).

In about 60% PCNSL localizes in the supratentorial space, which includes frontal (15%), temporal (8%), parietal (7%), occipital (3%) lobes, basal ganglia with the periventricular region (10%) and *corpus callosum* (5%), as well as 13% in the posterior fossa and about 1% in the spine. About 25-50% of PCNSL cases present with multiple formations (in patients with AIDS and after an organ transplant in 60-85%). Secondary PCNSL spreading to the brain layers is seen in 30-40% of the cases, but the development of a PCNSL in the leptomeninges was diagnosed only in 8% (15). Also PCNSL can be presented in the eyes because their formation is connected with CNS embryonic development and its frequency achieve 15-20% (21).

PCNSL presents mostly with focal neurological symptoms (50-80%), that depend on the localization of the tumor. That could be disorders of perception or movement, aphasia etc. Often patients have cognitive, behaviour and personality changes (20-30%), that associates with the tumor being in *corpus callosum* and in the frontal lobe. The symptoms of the increased intracranial pressure (10-30%) manifest as headache, nausea, optic disc oedema. Less often patients have seizures (5-20%) that are associated with the damage to the brain cortex. Vision problems (5-20%) manifest as monocular or binocular hazy eyesight, swimming or dashing elements in the eyesight, that can be associated with dynamic vitreoretinal traction during the posterior detachment of the vitreous body and the following vitreous hemorrhages (6,18,19,20).

In order to determine the localization and spread of a process in neuro-oncology as well as the level of damage the following methods are of most use: computer-tomography (CT), magnetic resonance imaging (MRI) and angiography. New methods of diagnostics appeared over the past 10-20 years, such as CT angiography, MRI angiography and venography, Single-photon emission computed tomography (SPECT), positron emission tomography (PET). These explorations help to early diagnose neoplasms in CNS and to apply treatment early, achieve better results in the treatment of neuro-oncological patients.

During the examination of the immunocompetent patient's, CT PCNSL appears as a periventricular or in the grey matter localized formation (18), that is hyperdense with a vasogene swelling, in MRI pictures the neoplasm is hypodense in T1 sequence (T1WI) and isointense or hyperintense in T2 sequence (T2WI). PET and SPECT are facultative methods of examination, that can help to differenciate PCNSL and others formations of the CNS in AIDS patients (12,32).

Morphological analysis is the main method to prove PCNSL diagnosis in neuro-oncological patients. During the microscopy of the PCNSL a massive lymphoid cell accumulation can be seen with the perivascular infiltrative damage and diffuse invasion of the parenchyma of the small arteria, arteriola and venules. In the periphery of the tumor a reaction of glia and infiltration of T-lymphocytes can be seen (see fig.4). Often there are isolated focuses of tumor cells near the primary tumor mass (16).

Histology shows the majority of PCNSL as typical B-cell non-Hodgkin lymphoma. The cells present with monotypical immunoglobulins, mostly IgM kappa, as well as with B-cell markers: CD19, CD20 and CD79a (28) (see fig.3). Based on Revised European-American Lymphoma (REAL) Classification and World Health Organisation (WHO) Classification 92-98% of all PCNSL are B-cell lymphomas (17). The incidence of a T-cell lymphoma is about 2-5%, but their number may be different depending on a geographical location, for example, the incidence of a T-cell lymphoma in Japan is 8-14% (21).

PCNSL are treated by combining different methods: neurosurgery, radiation, chemotherapy and corticosteroids. Corticosteroids may cause a formidable regression of PCNSL and immediate improvement of the clinical state (36). The lymphoma cells have receptors to glucocorticoids, that can cause cell apoptosis and decrease the size of the neoplasm in a few days after administration of corticosteroids and also decrease the vasogene swelling (22). Nevertheless the improvement is momentary and the tumor can retrieve its size in just a few months (12). Corticosteroids are not recommended in undiagnosed cases of PCNSL, because such therapy affects the results of biopsy and complicates diagnostics (23).

The aim of the neurosurgical manipulations is the reduction of the size of the neoplasm, that protects the brain from herniation and enhances the effect of the following chemotherapy, radiation and treatment with corticosteroids. Neurosurgical invasion can be used to acquire a biopsy that is one of the main elements of the diagnosis and planning of the following therapy (23).

There are two methods of treatment availiable in Radiation therapy of PCNSL: focal radiation and whole brain radiotherapy (WBRT). Focal radiation is applied to the constrained tumors of the brain, but its development in time is more often in comparison with WBRT (23), because PCNSL has an infiltrative growth pattern, so it is hard to define tumors borders. The results of the last researches show, that better outcomes are achieved using a focal therapy with larger area of radiation (4 cm) compared to using standard focal therapy (34). Autopsy data demonstrate that microscopical PCNSL focuses, that were not radiologically identified, are in multiple regions of the brain. So we can draw a conclusion that WBRT has more chances to control PCNSL development. The radiation dose is between 30 Gy and 50 Gy, but the optimal doses was not defined yet (4).

It is necessary to use such chemical agents in the chemotherapy of PCNSL, that can cross the hematoencephalic barrier in order to destroy the

tumor cells, that are not only around the blood vessels, but also deep in the brain tissues (29). Drugs, that are effectively used in the treatment of a systemic lymphoma are not effective in PCNSL, because they are unable to cross the hematoencephalic barrier (24). Some of the chemical agents and their combinations are used in treatment of the PCNSL, including CHOP and CHOD (cyclophosphamide, doxorubicin, vincristine, prednisolone or dexomethasone), but they give less effect compared to radiation therapy and have more toxicity (25). During the treatment with high dosis of methotrexate (MTX) as well as intrathecal MTX there were achieved much better results, the average length of life of the patients was up to 40 months, that is an indubious improvement compared to radiation (3,8,9). It is possible to achieve much better results and control of the disease by combining chemotherapy and radiation (23). Radiation of the brain and use of MTX can cause a later leucoencephalopathy, nevertheless there is an important clinical improvement at first. The consequences of the neurotoxicity appear in patients with longer survival. Patients develop a progressing dementia, ataxia, urine incontinence and memory loss. MTX is neurotoxic itself and can cause neurological symptomes such as: seizures, cognitive deficits, motor dysfunction. Intrathecal application can cause a chemical arachnoiditis that can manifest as acute meningitis (31,37).

AIM OF THE STUDY

PCNSL incidence amongst neurooncology patients is increasing in Latvia as well, therefore it is important to draw more attention to this problem. The aim of our study is to research and analyse the experience of the Riga Eastern Clinical University hospital (RECUH) in the management of the patients with PCNSL over the last 11 years (from 2001 till 2012). In this research, the data about the incidence of the disease, the connection between the age and sex, as well as the diagnostic and therapeutic possibilities and results are discussed.

MATERIAL AND METHODS

The research took part in the hospitals of RECUH: „Gailezers", „Latvian Oncology Center", „Linezers" and „Infectology Center of Latvia". In this research there are included patients, that were operated on and have morphologically confirmed PCNSL during the last 11 years (2001-2012). The number of patients in this 11 year period is 18. This is a retrospective descriptive study. The statistical analysis of the data was made by means of the Microsoft Excel 2010 and SPSS 20th version of the descriptive statistical methodes.

RESULTS

All patients (n=18) were included in the retrospective descriptive study, 17 (94.44%) of which were immunocompetent and in 1 (5.56%) HIV C1 stadium was diagnosed. Amongst immunocompetent patients 47.06% (n=8) were male and 52.94% (n=9) were female in the age between 45 and 79 years with average age of 64.41 years. Most often PCNSL was diagnosed in the age group between 65 and 69 years (see fig.1). There was one immunosupressed patient - a 29 years old male.

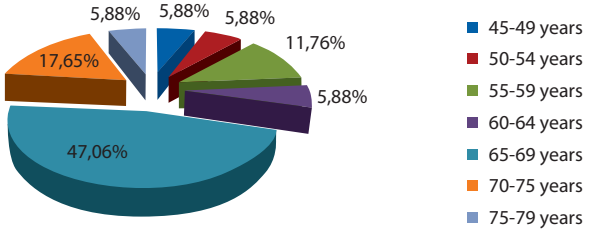

Fig. 1. PCNSL pacient`s age groups

While analysing the amount of the yearly diagnosed patients it can be seen that over the past 5 years there have been a heady increase of the number of patients along with the growth of the incidence compared to the previous years. 83.33% of all the PCNSL cases were diagnosed beginning with 2007, that is considered the begin of increase of lymphoma cases. The disease is piked in 2012 (n=4) and the incidence was 0.20 of 100 000 habitats per year (see fig.2).

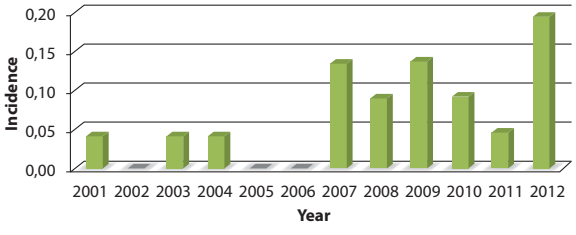

Fig. 2. PCNSL incidence of 100 000 habitats per year

In the diagnostics of all the patients (n=18) MRI was used as the imaging technique (see fig.5) and immunohistochemistry (CD5, CD20 as well as other cell markers if necessary) in order to morphologically confirm PCNSL (see fig. 3 and 4). Analysing of histological forms of PCNSL, in 100% of the cases was confirmed B-cell lymphoma (low-grade B-cell lymphoma (55.56%), diffuse large B-cell lymphoma (38.89%), intravascular B-cell lymphoma (5.56%)).

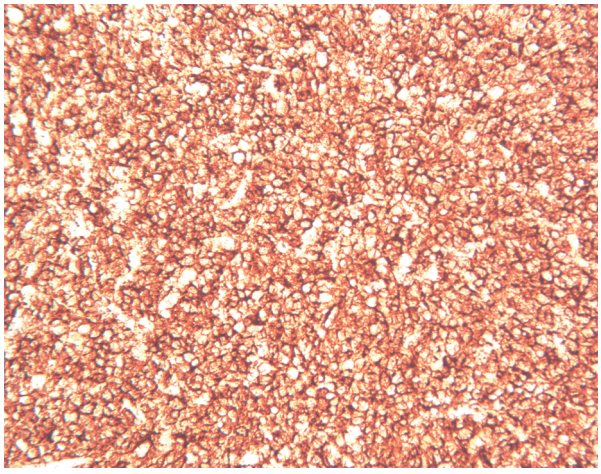

Fig. 3. Tumour cells express the pan-B-cell marker CD20. Obj. 20

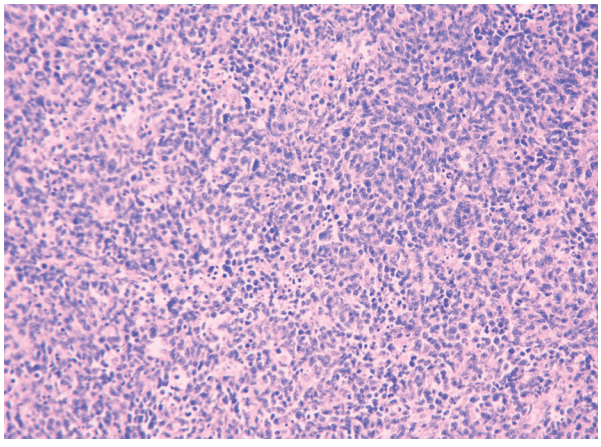

Fig. 4. Malignant, diffuse large B-cell lymphoma. Obj. 20

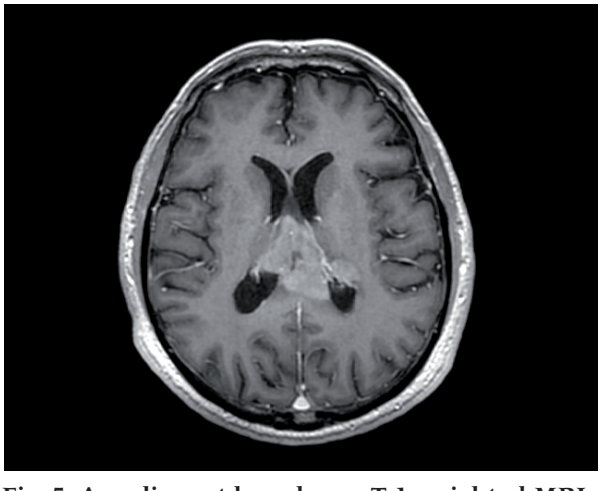

Fig. 5. A malignant lymphoma T-1 weighted MRI

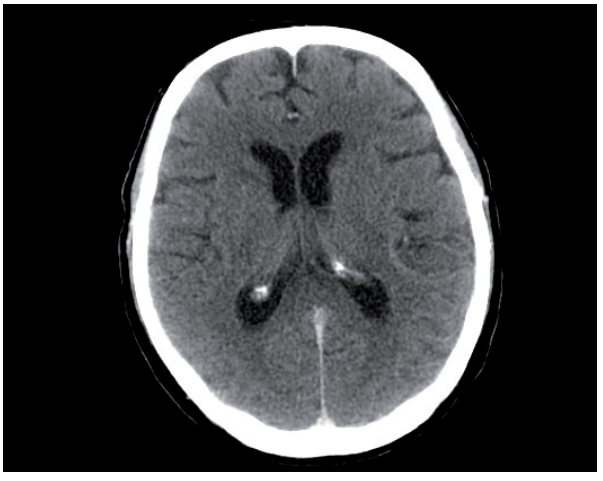

Fig. 6. Control-CT study view result after receiving a combination of therapy (the same patient)

The average Karnofsky Perfomance Scale (KPS) by admission was 57.78 amongst all the patients. 100% (n=18) of them received neurosurgical treatment, after which the KPS achieved 70. 12 patients were treated with radiation therapy, after which the KPS was by 72.50. Chemotherapy was applied to 8 patients, that achieved KPS 77.78 (see fig. 7).

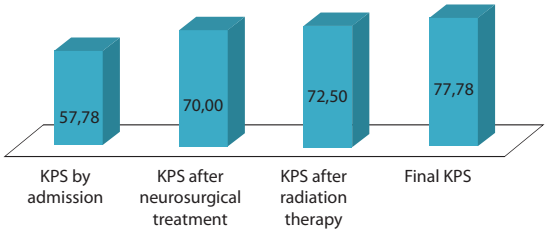

Fig. 7. KPS after receiving combined therapy stage

The prolonged development of the PCNSL was diagnosed in 22.22% (n=4) of the cases. Complications after neurosurgery were seen in 11.11% (n=2) as large ischemic stroke, but after radiation the complications reached 16.67% (n=2) – the patients had toxic-allergic reaction to radiation. The median survival amongst all the PCNSL patients was 515 days or 17 month, but patients that received combined therapy lived up to 867 days or 29 month (see fig.6).

DISCUSSION

Our study demonstrated that PCNSL is most often diagnosed in patients 65-69 years of age amongst immunocompentent patients and with mean age of 64.41 years (see fig.1), that agrees with the internationally published data, that says the most incidence occurs between 50-70 years of age with mean age of 60 years (24,33).

Our research demonstrated that the amount of PCNSL

patients has increased over the past 5 years, considering the yearly decrease of the country inhabitants, the incidence of PCNSL seems to be greater with the greatest index in 2012, when the incidence was 0.20 of 100 000 habitants (see fig.2). However, in comparison with the data described in the foreign literature, the incidence is less, that could be explained by the fact that not all PCNSL patients treated RECUH, some of them probably received necessary treatment in other medical institutions and so we do not reflect the incidence of the overall situation in society.

B-cell-lymphoma was morphologically confirmed in all the PCNSL patients, that is near to the results described in literature (92-98%), the discrepancy can be explained with our small amount of patients.

The PCNSL patients treated in RECUH received combined therapy consisting of neurosurgery, radiation and chemotherapy, after which the KPS index was 77.78 and the mean lifespan was 867 days or 29 month. Considering the starting KPS index (57.78) and the mean age of the patient (64.41 years) we think that the achieved results are good. The patients can take care of themselves and their quality of life is close to the normal level, as well as in the foreign literature describe the results of which PCNSL patients survival after received combination therapy ranges from 15 to 60 months (1,2,11,13,26,30), we can conclude that PCNSL patients treated RECUH survival approaching the average level of the world.

After the received therapy in the 22.22% (n=4) of the PCNSL patients the prolonged development of the tumor was stated, that is a large enough number and for that reason it is necessary to analyse the tactic of treatment in each stage, to find possible reasons and prevent them in the treatment of the following PCNSL patients. It is also necessary to analyse the reasons of the complications in order to possibly reduce its number. For example, it is sometimes better to take a larger biopsy during the neurosurgery than performing a subtotal evacuation.

In conclusion we want to add that even though PCNSL is a rare disease, its incidence and significance grows with every year in the modern society. In the risk group are the elderly and immunosupressed people, the number of which constantly grows in our country, that is why it is necessary to draw attention to this problem and find solutions. RECUH offers all the necessary methods of diagnosis and treatment in order to help the PCNSL patients to accept this diagnosis, improve the prognosis and the quality of life, but in order to achieve better results more detailed and extensive studies are needed as well as education of the medical attendants and the patients about PCNSL.

CONCLUSIONS

Most commonly PCNSL is diagnosed in the age range between 65 and 69 years, the mean age being 64.41 years and with sex proportion of 47.06% male and 52.94% females. Over the past five years the increase of the incidence appeared: 83.33% of all the PCNSL cases being diagnosed beginning with 2007, with greatest number of sick in 2012 (n=4), that is considered as an increase of lymphoma. The starting KPS index amongst all the PCNSL patients was 57.78, but after the combined treatment it increased up to 77.78, that shows considerable improvement. In all patients MRI was used as the imaging method, immunhistochemistry for morphological diagnosis of PCNSL and combined therapy, that consisted of: neurosurgery, radiation and chemotherapy, as a method of treatment and the median survival was 867 days or 29 months.

Conflict of interest: None

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ORIGINAL ARTICLE

TNF-α and IL-8 as Prognostic Markers of Birth Outcome in Overweight and Non-overweight Pregnant Women

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Summary
Introduction. Overweight has become a major risk factor for various diseases. Compared with normal weight overweight pregnant women have an increased risk of various complications in childbirth - invasive fetal monitoring, perineal ruptures, operative vaginal deliveries, cesarean sections, increased gestational age at delivery, and increased maternal length of stay are present. Both pregnancy and childbirth is a conditions significantly affected by the the immune system. However, this relationship and the possibility of its practical use is still not fully explored.
Aim of the study. Our aim is to determine whether overweight have the same impact on obstetrical outcome as obesity, explore changes of Interleukin-8 (IL-8) and Tumor Necrosis Factor-alpha (TNF-α) levels in the first and second trimester of pregnancy, compare these changes in normal weight pregnant women and pregnant women with overweight, to clarify their relationship to pregnancy outcome and to determine whether these cytokines have the potential to serve as biomarkers for prediction of labor complications.
Material and methods. This was a prospective, longitudinal study were we enrolled 55 pregnant women in their first antenatal visit. Blood samples were taken at different weeks of pregnancy. TNF-α and IL-8 concentrations were measured by Luminex xMAP technology (Luminex Corporation). Statistical analysis was performed using LibreOffice Calc, the Fisher exact test, Ttest, Spearman's rank correlation coefficient and a non-parametric Mann–Whitney–Wilcoxon test. For all statistical analyses, $p<0.05$ was considered statistically significant.
Results. Labor dysfunction and cesarean section were more frequently observed in women with high BMI. In cases of vaginal delivery ruptures were more often for patients in high BMI group but the difference is small. Although average birth weight was slightly lower in normal BMI group, the difference is not statistically significant ($p=0.13$). Male gender babies were more often for women with high BMI, but statistical difference is not significant ($p=0.15$). Comparing the levels of TNF-α and IL-8 in different weeks of pregnancy no statistically significant difference between the study groups was found. There was no strong tendencies in dynamics in TNF-α and IL-8 levels in differend first and second trimester weeks of pregnancy.
No statistically significant differences in levels of TNF- α and IL-8 depending on the fetal macrosomia were observed, but IL-8 level correlated with labour dysfunction and mode of delivery.
Conclusions. Overweight is a risk factor for labor dysfunction and probability of CS. TNF-α and IL-8 levels in early pregnancy does not differ in women with and without elevated BMI. In first and second trimesters of pregnancy no dynamics in TNF-α and IL-8 levels in maternal serum is observed. IL-8 level in the second trimester is correlated with labour dysfunction and increased cesarean section risk. This is an important finding, but its clinical value still requires further research.
Key words: maternal overweight; cytokines; TNF-α; IL-8; labor outcome.

INTRODUCTION

Overweight has become a major risk factor for various diseases. And concern is the fact that the number of people with elevated Body Mass Index (BMI) continues to grow. Obstetricians are increasingly confronted with elevated BMI patients and maternal obesity significantly contributes to a poorer prognosis for mother and baby during delivery and in the immediate post-partum period (22). Compared with normal weight overweight pregnant women have an increased risk of various complications in childbirth - invasive fetal monitoring, perineal ruptures, operative vaginal deliveries, caesarean sections (CS), increased gestational age at delivery, and increased maternal length of stay are present (5,6).

It has been found that pregnancy is a condition of moderate inflammation, although the physiological role of this low-grade inflammation remains unclear and also, obesity is a condition of chronic inflammation. This contributes to increased levels of circulating proinflammatory cytokines (21). There is evidence that the immune system is also important for the development of labor. Cytokines themselves might mediate the leukocyte attraction that occurs at the time of parturition. They might mediate leukocytic infiltration, either directly or via upregulation of cell adhesion molecules (26).
Tumor Necrosis Factor-alpha (TNF-*α)* is an inflammatory cytokine primary produced by macrophages and belongs

to the subpopulation of Th1 lymphocytes. In pregnancy it is also produced by placenta and membranes (4). This cytokine regulates a number of cell functions, including cell proliferation, differentiation, apoptosis and is involved in the metabolic regulation of glucose, lipids, and insulin resistance (18,4). Studies have confirmed that increase of TNF-α, is related to the risk for developing obstetric complications, particularly recurrent fetal loss, GDM, hypertensive syndromes, and fetal growth restriction, but these results remain controversial (2). And there are limited data on the early pregnancy.

Interleukin-8 (IL-8) is a member of CXC chemokine subfamily it is responsible for the recruitment of monocytes and neutrophils thus engaging in inflammatory response (1). In terms of pregnancy the relationship has been found that risk of spontaneous preterm delivery and preeclampsia is increased in women with high IL-8 levels (20,3,23). However, there is no conclusive data on the early pregnancy cytokine levels and birth outcome.

AIM OF THE STUDY

We wanted to determine whether excess weight have an impact on obstetrical outcome.

Objective of this study is to explore IL-8 and TNF-α level changes in the first and second trimester of pregnancy, compare these changes in normal weight pregnant women and pregnant women with overweight and to clarify their relationship to pregnancy outcome. The aim is to determine whether these cytokines have the potential to serve as biomarkers for prediction of labor complications.

MATERIAL AND METHODS

This was a prospective longitudinal study in which we included women who received perinatal care at Riga Maternity Hospital Outpatient Department and gave birth at Riga Maternity Hospital in years 2011th and 2012th. Pregnant women were recruited at their first antenatal visit at 9-12 weeks of pregnancy. The study included healthy, adult pregnant women without history of previous adverse obstetrical outcome and CS. Exclusion criteria were serious illnesses - cardiovascular, respiratory, endocrine abnormalities, allergies and smoking.

All the women recruited were measured for weight and height, and the BMI was calculated. Two groups of patients were defined according to the result: one group with excessive weight (defined as BMI above 24.9 kg/m²) and a second group with normal BMI

55 women were enrolled, 17 in group with excess weight (average BMI 28.81 kg/m² - 11 overweight(BMI from 24.9-29.9 kg/m²) and 6 obese (BMI from 30 kg/m²)) and 38 in group with normal BMI (average BMI 21.69 kg/m²).

Further for women from both groups blood samples were taken in their first visit and in visits at 15-18 and 24-28 weeks gestation.

Following delivery main clinical characteristics were collected and included to the analyses: mode of delivery, indication for CS(labor dysfunction, fetal distress, cephalopelvic disproportion, placental pathologies), complications of vaginal delivery (labor dysfunction, fetal distress, ruptures during childbirth- any degree cervical, vaginal or perineal lesions which were diagnosed and required suturing), gestational age at delivery, birth weight and gender.

The study was approved by the Latvian University Ethics Committee. A written informed consent was obtained from each patient.

Blood samples were collected from an antecubital vein after a 12-h fast.

The samples were allowed to coagulate for 20 to 30 min at room temperature. Sera were separated by centrifugation at 4 °C for 20 min at 1600×g. All specimens were immediately aliquoted, frozen, and stored at–80°. TNF-α and IL-8 concentrations were measured by Luminex xMAP technology (Luminex Corporation).

Statistical analysis was performed using LibreOffice Calc. Data are reported as percentages for categorical variables and as median for continuous variables. The Fisher exact test was used to compare categorical variables and Ttest to compare continuous variables between groups. Spearman's rank correlation coefficient (Spearman's rho) was applied to assess relationship between cytokines and BMI. A non-parametric Mann–Whitney–Wilcoxon test was used for evaluation between TNF-α and IL-8 levels and fetal macrosomia and obstetrical outcome. For all statistical analyses, $p<0.05$ was considered statistically significant.

RESULTS

Differences were found in the outcome of pregnancy in women with and without excess weight.

Obstetrical outcome of the study population are shown in Table 1.

Table 1. Obstetrical outcome characteristics of the study groups

	High BMI (n=17)	Normal BMI (n=38)	P-value
Age (mean number)	29.94 (21-40)	28.89 (19-38)	0.49
Gravidity (mean number)	2.11 (1-5)	2.26 (1-5)	0.68
Parity (mean number)	1.71 (1-4)	1.84 (1-4)	0.6
Gestational age (weeks)	39.59 (37-41)	39.53 (38-41)	0.83
Caesarean Section, % (n)	47 (8)	8 (3)	0.002
Labour dysfunction, % (n)	43 (6 of 14)	8 (3 of 36)	0.01

	High BMI (n=17)	Normal BMI (n=38)	P-value
Ruptures in childbirth, % (n)	57 (8 of 14)	50 (15 of 30)	0.75
Birth weight (g)	3954 (3360-4320)	3754 (2730-5130)	0.13
Male gender, % (n)	65 (11)	42 (16)	0.15

No significant difference in terms of age, gravidity, parity and gestational age among study groups were found. But large differences were observed in the mode of delivery. Cesarean section (due to various reasons - dysfunction, fetal distress, cephalopelvic disproportion or placental pathologies) were more frequently applied to women with high BMI (47% vs 8%; p=0.002). Labor dysfunction had 43% women of high BMI group and 8% women of normal BMI group (p=0.01). In cases of vaginal delivery ruptures were more often for patients in high BMI group but the difference is small (p=0.75). Although average birth weight was 3954g in high BMI group and 3754g in normal BMI group, the difference is not statistically significant (p=0.13). Male gender babies were more often for women with high BMI, but statistical difference is not significant (65% vs. 42%; p=0.15).

To exclude the possibility that birth complications in the group with excessive weight is more due to obese women, we compared data about adverse obstetrical outcome inside the group - between overweight and adipose women. The results, which we obtained showed that the percentage of complications was similar in both subgroups. Level of statistical significance of any of the parameter was not p<0.05. Most notable difference was found in terms of risk of rupture in labor - 36.36% vs. 66.67%, however, because of a small number of patients in each subgroup, this correlation can not be considered statistically significant (p=0.33).

Serum levels (pg/ml) of cytokines in pregnant women with high and normal BMI at different weeks of gestation are shown in Table 2.

Table 2. Cytokine levels in pregnant women with high and normal BMI

	High BMI	Normal BMI	Spearman's rho	P-value
TNF-α at 9-12 weeks of gestation (pg/ml)	8.4	8.2	0.076	0,65
TNF-α at 15-18 weeks of gestation (pg/ml)	8.1	9.4	-0.051	0.76
TNF-α at 24-28 weeks of gestation (pg/ml)	9.0	8.5	-0,001	1.00
IL-8 at 9-12 weeks of gestation (pg/ml)	27.2	28.9	0.049	0.79
IL-8 at 15-18 weeks of gestation (pg/ml)	22.7	55.5	0.225	0.25
IL-8 at 24-28 weeks of gestation (pg/ml)	26.5	19.8	0.001	1.00

Comparing the levels of TNF-α and IL-8 in different weeks of pregnancy no statistically significant difference between the study groups of overweight and pregnant women with normal BMI was found.

Dynamic of TNF-α and IL-8 values in first and second trimester of pregnancy is displayed in Figures 1-6.

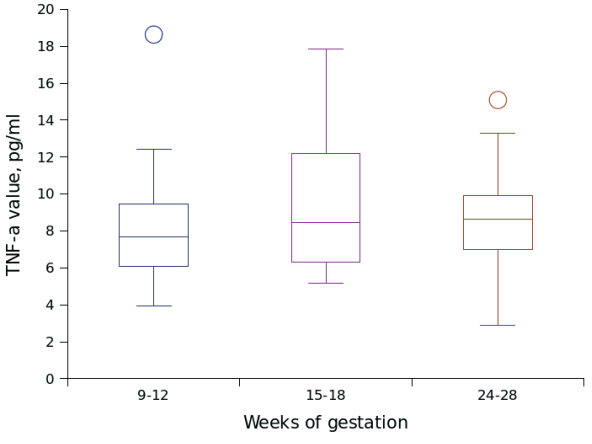

Fig. 1: TNF-α values of normal BMI patients

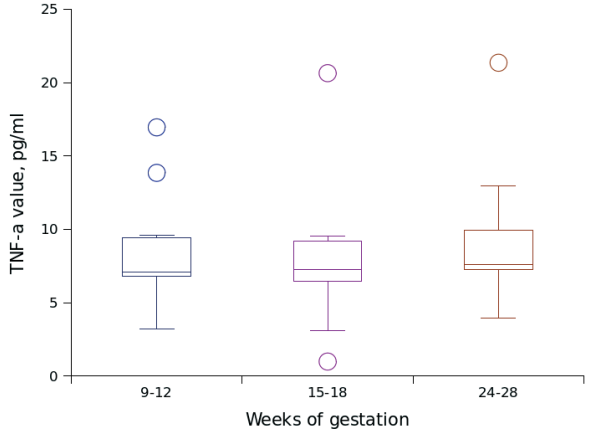

Fig. 2: TNF-α values of high BMI patients

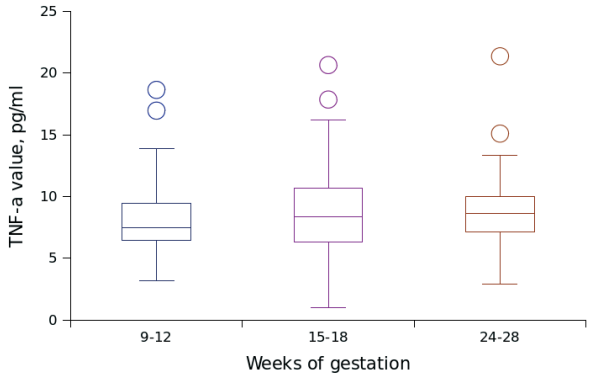

Fig. 3: TNF-α values of all patients

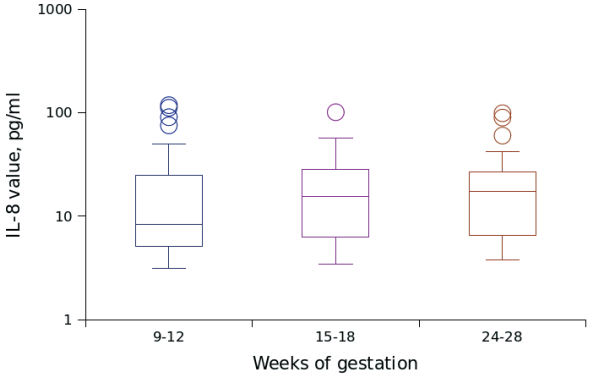

Fig. 4 IL-8 values of normal BMI patients

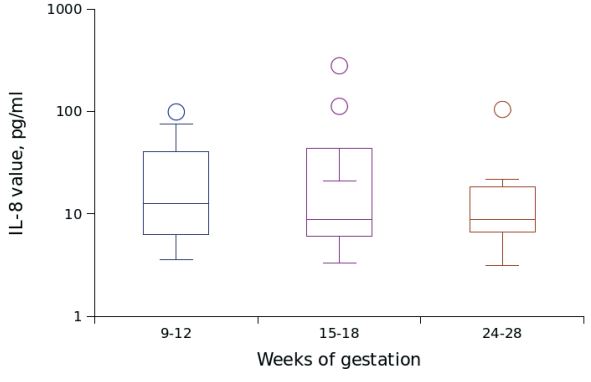

Fig. 5 IL-8 values of high BMI patients

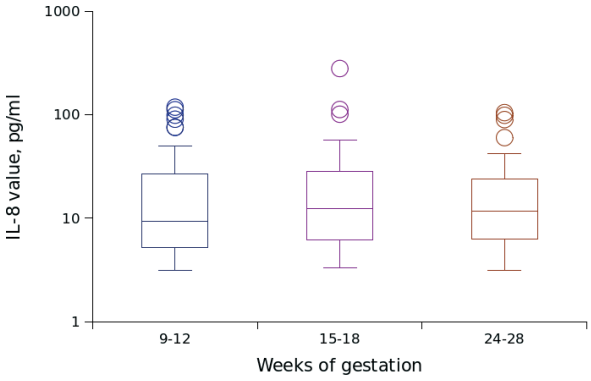

Fig. 6 IL-8 values of all patients

There were no strong tendencies in dynamics in TNF-α and IL-8 levels in different first and second trimester weeks of pregnancy.

No statistically significant differences in levels of TNF- α and IL-8 depending on the fetal macrosomia were observed, but IL-8 level correlated with labor dysfunction and mode of delivery. Statistical reliability is shown in Table3.

Table 3. The statistical significance of the relationship between cytokine levels and labor outcomes- results of Wilcoxon test

	TNF-alpha at 9-12 weeks of ges-tation	TNF-alpha at 15-18 weeks of ges-tation	TNF-alpha at 24-28 weeks of ges-tation	IL-8 at 9-12 weeks of ges-tation	IL-8 at 15-18 weeks of ges-tation	IL-8 at 24-28 weeks of ges-tation
Fetal macro-somia	W = 134.5, p-value = 1	W = 158, p-value = 0.5618	W = 133.5, p-value = 0.4398	W = 79, p-value = 0.6201	W = 93, p-value = 0.5327	W = 78, p-value = 0.5295
Labor dysfun-ction	W = 64, p-value = 0.957	W = 96, p-value = 0.01899	W = 52, p-value = 0.3881	W = 64, p-value = 0.957	W = 96, p-value = 0.01899	W = 52, p-value = 0.3881
Mode of deli-very	W = 143.5, p-value = 0.5087	W = 50, p-value = 0.9138	= 74, p-value = 0.07043	W = 68, p-value = 0.1897	W = 42, p-value = 0.7796	W = 98, p-value = 0.03875

DISCUSSION

In this study, we wanted determine whether there is the possibility to find biomarkers that could help to predict obstetric complications in early pregnancy. We chose two cytokines from different groups in order to to test their level in maternal serum and to find out whether there are differences depending on the BMI and on obstetric outcome.

Most studies have analyzed pregnant women with obesity but to situation in Latvia much greater importance would be if specified role of excessive weight in the development of obstetric complications would be

established. Therefore, in this study we chose to split patients with and without high BMI. And our study confirmed that increased weight itself is a risk factor for birth dysfunction and higher rate of CS. Similar results were also obtained in other studies, but in those obesity was analyzed as a risk factor (6,24). A large retrospective cohort study demonstrated that elevated prepregnancy weight increase the risk of prolonged and postterm delivery (41 or 42 weeks) (10). In our prospective study we did not find a significant difference at the pregnancy solution time. Mothers who are obese have an increased risk of tears during childbirth (16). In our study that was not confirmed, but it can be explained by the fact that CS outweigh the vaginal birth for women with a high BMI. Studies in which fetal weight is compared between pregnant women with and without obesity show that it is significantly larger in cases of obesity (25). In our research we found slightly higher fetal weight in women with higher BMI, but the difference was not statistically significant. This suggests that the excessive weight do not affect the child's risk of macrosomia.

In terms of cytokine level changes during pregnancy our study showed no significant changes in TNF-alpha and IL-8 levels in maternal serum. Other longitudinal studies have found an increase of TNF-alpha in late (34-36 weeks) pregnancy (15). This is explained by the fact that the placenta were shown to express TNF and its receptors is with greatest activity evident in third trimester which explains the increase in TNF-α levels (9). Also IL-8 levels did not change in pregnancy, they rose just in labour (11).

We found no correlation between TNF-α and maternal BMI. Our findings are similar to results of study were women had their inflammatory markers measured at term before labour (17).

In another study correlation has been found between IL-8 and maternal adiposity, but samples were taken and cytokines were measured only at 28 and 37 weeks gestation so there is no clarity on the situation in early pregnancy (8). According to results of our research IL-8 levels during early pregnancy does not depend on pre-pregnancy BMI.

Also we found that maternal TNF-α and IL-8 levels in first and second trimester do not have relationship with fetal macrosomia. Similar results were also obtained in researches were IL-8 and TNF-α levels were analyzed in cord blood samples and in maternal blood samples taken in second half of pregnancy (18,8). TNF-α also did not correlated with labor dysfunction and CS risk. This is consistent with the results which are obtained in study were material from 55 patients were taken with blood sampling from a clamped segment of cord after delivery of the fetus and from the cord at its insertion into the placenta after delivery of the placenta. TNF-α levels for both patient groups were uniformly low for all of the cord measurements with no significant differences noted (7).

There are few studies to look for correlations between cytokine levels in early pregnancy and mode of delivery. So far, IL-8 role as a biomarker the most have been studied in relation to risk of premature birth (12,13). Our results show that IL-8 levels in the second trimester is correlated with birth dysfunction and thus the risk of cesarean sections. This is a very important discovery, which still requires further research. However, this could be due to the valuable role played by immune system in childbirth. Also we know that IL-8 increases activity of collagenase and metalloproteinases 8 and 9, which are required for softening of cervical tissue what is very important during childbirth (14). These factors could be decisive in the development of complications in labor and thus affect the need for SC. Our study shows that there are changes that exist in a relatively early time of pregnancy. Of course, larger studies with greater sub-groups in order to clarify the prognostic role of cytokine in the development of complications in labor is necessary.

CONCLUSIONS

1. Not only obesity, but also excessive weight altogether is a risk factor for labor dysfunction and probability of CS.
2. TNF-α and IL-8 levels in early pregnancy does not differ in women with and without elevated BMI.
3. In first and second trimesters of pregnancy no dynamics in TNF-α and IL-8 levels in maternal serum is observed.
4. IL-8 level in the second trimester is correlated with labour dysfunction and increased cesarean section risk. This is an important finding, but its clinical value still requires further research.

Conflict of interest: None

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ORIGINAL ARTICLE

Variations in Lower Limb Deep Venous Anatomy in Latvia

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Summary
Introduction. During the last several decades there have been many new methods introduced for the treatment of lower limb chronic venous insufficiency (laser, foam, subendothelial and thermal coagulation methods). Venous system of lower limbs often presents anatomic variations including venous duplications. Knowledge of venous system variations in the lower limb area is of particular importance due to correct interpretation of imaging in relation to deep vein thrombosis (DVT). There was only a small number of studies published on anatomic variations of venous system in the lower limbs. To the best of our knowledge there have been no previous studies on anatomic variations of lower limb deep venous system performed in Latvia.
Aim of the study. To retrospectively review of 216 patients (432 lower limbs) phlebograms in order to establish deep venous system anatomic variations in Latvian population and compare our results to other publications.
Materials and methods. Retrospective analysis of 432 lower limb phlebograms performed at Pauls Stradins Clinical University Hospital (Riga, Latvia) of 216 patients treated in different ortopedic centers of Latvia during 2009 and 2012. Assessment made using DICOM Synedra view personal software. Study protocol was developed for definition of veins and assessment of phlebogram images in accordance with anatomic definitions used in previous studies. Two independent radiologists assessed data. Visualised duplications in the deep venous system of both lower limbs in patients were registered (common iliac vein, external iliac vein, common femoral vein, femoral vein, deep femoral vein, popliteal vein). Blood vessels have been listed as single, double or triple / complex. The presence of DVT was recorded upon assessment of phlebograms. Statistical analysis performed using SPSS 20.0 software (IBM). Parametric data comparison performed using Student t-test and ANOVA. Non-parametric data comparison performed using chi-square and Mann Whitney tests. Data comparison type was assessed using Kolmogorova-Smirnovs test. The results are presented as the average $\pm$ standard deviation.
Results. Retrospective analysis of 432 lower limb phlebograms was performed in 216 patients. Average age of the patients was 34.4 years (range 19-90). 101 patients were female (47%) and 115 (53%) were male with no statistical venous variation differences found between two genders, which is explained by both age and gender ($p > 0.05$). Analysis of calf vein, popliteal vein and femoral venous variations provided a strong correlation between larger number of duplications in one limb and possibility of such variations in the other limb of the same patient (all $p < 0.001$).
Conclusion. We conclude that there are frequent anatomic variations in SFV and popliteal veins seen in Latvian population. All patients included in this study had high DVT risk, much higher than in the average Latvian population. In almost every sixth Latvian person there is some form of deep veins hypoplasia found.
Keywords: venous anatomy; deep veins; femoral vein; popliteal vein; calf veins; venography.

INTRODUCTION

Up to 73% of women and 53% of men worldwide are diagnosed with chronic venous insufficiency (CVI). In Europe over one third of population is diagnosed with CVI, 40-60% of females and 15-30% of males (5). Furthermore, deep venous thrombosis (DVT) occurs for the first time in about 100 persons per 100 000 each year (6). Precise diagnosis and proper treatment reduce morbidity and mortality from DVT. Therefore it is essential to understand the normal anatomy of lower limb venous system in order to establish correct diagnosis and provide treatment. There are frequent variations found in deep veins of lower limbs. Duplications of femoral veins may be one of the potential reasons for wrong DVT diagnosis.

The significance of lower limb normal venous anatomy and its variations was determined in line with the development of minimally invasive surgical interventions methods for the treatment of lower limb chronic venous insufficiency (laser, foam, subendothelial and thermal coagulation methods). The understanding of lower limb normal anatomy changes is important in order to prevent unforeseen complications in this region during intervention.

Veins serve as the best material for arterial grafting and therefore the understanding of deep and superficial veins condition is especially important. Apart from that sometimes the duplication of femoral vein is used in arterial reconstructions if large saphenous vein is not available.

There is a small number of studies published on anatomic variations in venous system. *Quinlan et al* report that the incidence of femoral veins' duplications is approximately 20-25%, however this incidence could

be higher if also partial duplications were taken into account (5).
Until now there have been no studies on lower limb deep venous system anatomic variations performed in Latvia. The aim of this study was retrospective analysis of lower limb deep venous system variations in Latvia and comparison of results to published data on other populations.

MATERIALS AND METHODS

Retrospective analysis of lower limb phlebograms in 216 patients (432 limbs) was performed. Phlebographies were performed in the period between 2009 and 2012 in Pauls Stradins Clinical University Hospital (Riga, Latvia). All patients have undergone hip or knee endoprosthetic replacement and received prophylactic anticoagulant therapy in different ortopedic clinics in Latvia. The subiect group represents unselected patients proportionally coming from all regions in Latvia. Phlebography was performed in order to exclude DVT, which is an often complication after joint replacement surgery. Phlebograms of 15 patients have been excluded from the study due to poor imaging quality preventing from assessing venous anatomy.
Phlebograms were assessed using DICOM Synedra view personal software. Prior to commencing the study there was a study protocol developed for definition of veins and assessment of phlebogram images in accordance with anatomic definitions used in previous studies. Two independent radiologists assessed data. In case of discordance of the results between them, patient was re-evaluated and final conclusion made.
Visualised duplications in the deep venous system of both lower limbs in patients was registered (common iliac vein, external iliac vein, common femoral vein, femoral vein, deep femoral vein, popliteal vein). Blood vessels have been listed as single, double or triple / complex.
In-flow point (junction) of every limb's vein into the calf or popliteal vein and the location of this junction with respect to other vessels was marked. Using this information the classification was made defined as calf's veins join in trifurcation before creating a popliteal vein. Hypoplasticity of calf's deep veins was also marked. Hypoplastic veins were defined as those with diameter <50% from other calf's deep veins diameter.
Number of vessels in popliteal fossa have been assessed and counted, including those vessels crossing the knee joint. Using popliteal fossa as the landmark, the location of popliteal vein creation was recorded. Duplications and 'true' duplication of popliteal vein were recorded. The 'true' popliteal vein duplication is when popliteal vein commences and finishes in the popliteal fossa region. Popliteal vein was classified as single, double or triple.
Femoral vein (FV) was registered as single, double or triple /complex. Special attention was paid to analysis of deep femoral vein and large saphenous vein in order to avoid wrong interpretation of the aforesaid veins as FV. FV duplications were assed at the point of their origin, position, length and size with respect to the original FV that was defined as the vessel that most closely followed the course of the superficial femoral artery. Position of FV duplication was registered as medial or lateral against the true FV, or both, if such duplications were from both sides of the true FV. Lengths of FV duplications were divided into the following length groups: 1-5 cm, 6-10 cm, 11-20 cm, 21-30 cm and 31 cm or larger. In addition to that the level of lowest duplication point was recorded: above or in adductor channel region, above or under patella. Each direct FV and deep femoral vein junctions with distal anastomoses have been recorded.
The presence of DVT was recorded upon assessment of phlebograms.
Statistical analysis performed using SPSS 20.0 software (IBM). Parametric data comparison performed using Student t-test and ANOVA. Non-parametric data comparison performed using chi-square and Mann Whitney tests. Data comparison type was assessed using Kolmogorova-Smirnovs test. The results are presented as the average ± standard deviation.

RESULTS

Retrospective analysis of 432 lower limb phlebograms was performed in 216 patients. Average age of the patients was 34.4 years (range 19-90). Out of all patients 101 patients were female (47%) and 115 (53%) were male with no statistical venous variation differences found between two genders, which is explained by both age and gender ($p > 0.05$). Analysis of calf vein, popliteal vein and femoral venous variations provided a strong correlation between larger number of duplications in one limb and possibility of such variations in the other limb of the same patient (all $p < 0.001$).
The majority of anterior tibial veins and posterior tibial veins and peroneal veins were paired – respectively: 71% (307 and 432), 90% (388 and 432) and 89% (384 and 432). Single veins were seen in 26% (111 our of 432), 9% (39 out of 432) and 5% (23 out of 432) cases respectively. Three or more peroneal veins were found in 6% (25 out of 432), part of anterior tibial veins in 3% (14 out of 432), posterior tibial veins in 1% (5 out of 432) patients. Drainage of the peroneal veins into the trifurcation occurred in 67% (288 out of 432) of cases; into the posterior tibial vein in 25% (110 out of 432) cases and into anterior tibial veins in 8% (34 out of 432) cases respectively. Gastrocnemius vein were only visible in 60% (259 out of 432) cases, and 78% (202 out of 259) cases the drainage was above the knee joint. A variable number of gastrocnemius veins (one to six) were visualised.
In the venous system of the calf 18% (79 out of 432 lower limbs) of veins were hypoplastic. Out of these 11% (49 out of 432 lower limbs) had hypoplastic anterior tibial veins, 6% (27 out of 432 lower limbs) posterior tibial veins and 0.7% (3 out of 432 lower limbs) peroneal veins. The incidence of hypoplastic veins did not differ between right and left limbs, between gender and age of the patients (all $p>0.05$). In neither of the limbs we have visualised venous agenesis. Most frequently (49 out of 79 hypoplastic veins) anterior tibial vein hypoplasticity

was diagnosed. However hypoplastic peroneal veins were visualised only in three patients (Table 1 and 2).
Data on popliteal vein are presented in Table 3. In total there have been 51% (220 out of 432 limbs) popliteal vein true and false duplications visualised. Out of these 176 were duplications, whereas five triple or complex. Jointly 181 (42%) popliteal vein duplications were diagnosed, out of which true duplications recorded in 39 (9%) out of 432 lower limbs. The length of duplicated popliteal vein in both limbs was ranging from 6.0 up to almost more than 8.9 cm with average length being 7.5±6.9 cm. Length of duplications in the right and left limb as well as intra-gender differences have been significantly different ($p<0.05$). The origin of popliteal vein at popliteal fossa level was found in only six (1%) of patients' limbs, proximally from fossa in 269 (62%) of cases and distally from the knee joint in 157 (36%) out of 432 lower limbs. Within a popliteal fossa a single vessel was identified in 47 (11%) of phlebograms, two vessels in 307 (71%) phlebograms, three or more vessels in 78 (18%) out of 432 lower limbs. The incidence of popliteal veins did not differ between patients' age and gender ($p>0.05$). However there was a statistically significant incidence of true popliteal vein duplication found between patients' age and gender ($p<0.05$). Full details provided in Table 4.
FV duplications were found in 188 (43%) out of 432 limbs, out of which the majority 38% (166 out of 188) were double (Figure 1b). The remaining 5% (22 out of 432) had more complex venous system. Complex femoral veins provided in Figure 1a. Medial duplications (Figure 2a) found in 120 (63%) of 188 duplicated vessels, out of which 74 (39%) originated in the middle of the thigh (above adductor channel) and 68 (36%) originated in the adductor channel area. The length of duplicated femoral veins in both limbs differed from 1 up to more than 30 cm, with average length 14.9 ± 6.1 cm (standard deviation): 13.5 ± 6.0 cm duplicated and 16.5 ± 7.4 cm complex duplicated veins. More details can be found in Tables 4 and 5. In cases of complex venous segment (triple/complex) these were longer than double veins ($p < 0.05$ in both limbs). In comparison to duplications, complex veins were located in adductor channel projection ($p < 0.05$ for both limbs). Out of 188 femoral veins duplications 122 (65%) femoral vein diameters were smaller than one third of the main femoral vein, but 53 (28%) were half the diameter. In only small number of cases, that is 13 out of 188 (7%) was the duplicated FV the same size. Most frequently in 32% of cases (130 out of 432 limbs) there was FV seen without duplication with popliteal vein originating from the knee joint level (Figure 3a). Out of FV duplication types 19% (83 out of 432 limbs) FV duplications were found with popliteal veins originating proximally from the knee joint. Most frequently found popliteal vein variations were true duplications (5%) originating and ending in popliteal fossa region (Figure 3.O).
Axial transformation of FV (Figure 2b) was found in 22% (95 out of 432 limbs) cases. No duplications were found in common iliac veins and deep femoral veins. However in three (0.7%) patient limbs external iliac vein duplications were found. All three external iliac vein duplications were located in the right limb. Common femoral vein duplications were found in 11 (3.5%) cases in both limbs. Seven duplications were located in the right limb and four in the left limb (Tables 6 and 7). On comparison of common femoral vein duplications between right and left patient limbs it was found that the incidence of common femoral vein duplications is significantly different between two limbs ($p=0.008$).
Upon assessment of phlebograms DVT was found in 6% (24 out of 432) cases. Right limbs of the patients had DVT in 11 (5%) of phlebograms analysed and left limbs had DVT in 13 phlebograms (6%). No significant difference found between in gender prevalence ($p=0.266$) (Table 6).

DISCUSSION

There was a significant range in the length of duplicated veins. The lengths of duplications did not differ on average in terms of the size, both on comparison of patients' limbs and gender differences. Only popliteal vein short duplications lengths have shown significant differences on comparison between genders and right and left limbs. In our study the dominant limb of the patient was the left limb, which was found to have significant differences in longer duplications. Similar findings were published in the study of *Park et al*.
Hypoplastic calf veins were found comparatively more often (18%). We compared the studies of *Eifert* and their findings have significantly differed from our results, where 392 patients were studied and 8% of calf veins were found to be hypoplastic. This study has included patients with already diagnosed congenital vascular malformations (1).
Data provided by our study also differs from *Quinlan et al* results (5), where they report the majority (65%) of cases in popliteal vein duplications lengths to originate distally from the knee joint. In our study we found that in 62% of cases popliteal vein was originating proximally from the knee joint, which is similar to the findings of *Park et al* (5). Such differences could again be explained by different study methodology, where, for example, *Quinlan et al* study analysed only the phlebograms of the patients participating in enoxaparin study (MEDENOX) (5). All patients with a history of DVT were excluded from the study. In the study of *Park et al* the analysis of venous system was performed using computer tomography images and not phlebograms. This study included patients with varicose veins complications and the analysis was made based on perioperative imaging studies (4).
An important hypothesis is whether duplication is a separate DVT risk factor. In our study in non-duplicated limbs there have been only 6% (24 out of 432) DVT cases recorded. This number is different from the previously published data of other authors. *Liu et al* study has recorded DVT in 19% of patients (3). In our study and also other published studies the hypothesis of

increased DVT risk at auxiliary diagnosed duplications was not confirmed. Different methodology of our study could have influenced this result. In the study performed by *Liu et al* there were patients with high DVT risk analysed however symptomatic DVT patients were not excluded from the study (3). As a bias for the study authors should point out fact, that all patients included in this study had high DVT risk, much higher than in the average Latvian population due to orthopaedic surgery they underwent (7).

CONCLUSIONS

We conclude that there are frequent anatomic variations in FV and popliteal veins seen in Latvian population. In almost every sixth Latvian person there is some form of deep veins hypoplasia found.

Conflict of interest: None

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Table 1. Comparison of hypoplastic deep veins in the right and left calf of patients

Hypoplastic Vein	Right Limb	Left Limb	Both Limbs (n = 432)
Anterior tibial veins	20	29	49 (11%); p>0.05
Posterior tibial veins	12	15	27 (6%); p>0.05
Peroneal veins	2	1	3 (0,7%); p>0.05
Total	34	45	79 (18%); p>0.05

Table 2. Incidence of hypoplastic calf veins in different genders

Hypoplastic vein	Females	Males	Total (n=432)
Anterior tibial veins	21	28	49 (11%); p > 0.05
Posterior tibial veins	13	14	27 (6%); p > 0.05
Peroneal veins	1	2	3 (0,7%); p > 0.05
Total	35	44	79 (18%); p > 0.05

Table 3. Origin of popliteal vein and number of vessels in popliteal fossa

Origin of popliteal vein			
At knee joint level	5	1	6 (1%) p>0.05
Proximally from knee joint	137	132	269 (62%) p>0.05
Distally from knee joint	74	83	157 (36%) p>0.05
Number of vessels in popliteal fossa region			
Single	27	20	47 (11%); p>0.05
Double	152	155	307 (71%); p>0.05
Three or more	37	41	78 (18%); p>0.05
True popliteal vein duplication	17	22	39 (9%); p<0.05

Table 4. Average length of FV and popliteal vein duplications in different limbs

Length of duplications (cm)	Right Limb	Left Limb	Both Limbs (n = 432)
Popliteal vein duplication	7.4 ± 0.7	7.6 ± 0.7	7.5 ± 0.7; p > 0.05
True popliteal vein duplication	3.2 ± 0.5	4.2 ± 0.8	4.0 ± 0.6; p < 0.05
FV duplication	14.3 ± 6.1	13.6 ± 6.2	13.5 ± 6.0; p > 0.05
True FV duplication	19.1 ± 6.4	16.0 ± 6.5	16.5 ± 7.4; p < 0.05

Table 5. Position, length and lowest point of FV duplication

FV	Right Limb	Left Limb	Both Limbs
Position			
Medial	53	59	120 (63%); p > 0.05
Lateral	32	33	56 (30%); p > 0.05
Both	9	3	12 (6%); p > 0.05
Length (cm)			
1-5	4	6	10 (5%); p > 0.05
6-10	22	31	53 (28%); p > 0.05
11-20	52	44	96 (51%); p > 0.05
21-30	15	12	27 (14%); p > 0.05
>30	1	1	2 (1%); p > 0.05
Lowest point			
Under patella	2	1	3 (2%); p > 0.05
Above patella	26	17	43 (23%); p > 0.05
In adductor channel	25	43	68 (36%); p < 0.05
Above adductor channel	40	34	74 (39%); p < 0.05

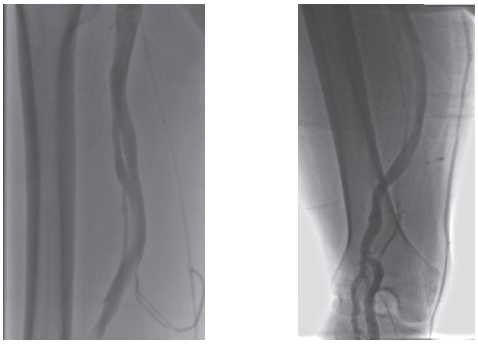
Table 6. Incidence of deep vein duplications in different limbs

Duplication	Right Limb	Left Limb	Both Limbs (n=432)
Common iliac vein	0	0	0
External iliac vein	3	0	3 (0.7%); p < 0.05
Common femoral vein	7	4	11 (2.5%); p < 0.05
Femoral vein	94	94	188 (43.5%); p > 0.05
Deep femoral vein	0	0	0
Popliteal vein	88	93	181 (42%); p > 0.05
True popliteal vein duplication	17	22	39 (9%); p < 0.05

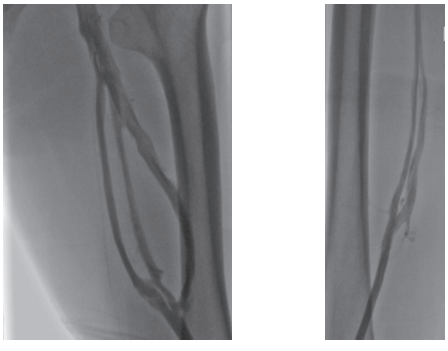
Table 7. Duplications of deep veins in different genders

Duplications	Females	Males	Total (n=432)
Common iliac vein	0	0	0
External iliac vein	2	1	3 (0.7%); p > 0.05
Common femoral vein	7	4	11 (2.5%); p < 0.05
Femoral vein	82	106	188 (43.5%); p > 0.05
Deep femoral vein	0	0	0
Popliteal vein	86	95	181 (42%); p > 0.05
True popliteal vein duplication	23	16	39 (9%); p < 0.05

Table 8. Isolated FV duplications and DVT



a) *b)*



a) *b)*

Fig. 1. a) Complex femoral vein duplication; b) Simultaneous duplication of femoral and popliteal veins

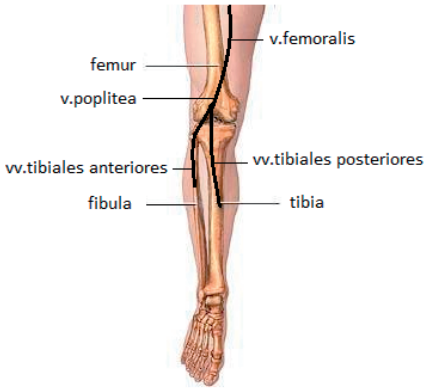


Fig. 2. a) Medial duplication of FV; b) Axial transformation of deep femoral vein and duplication of FV

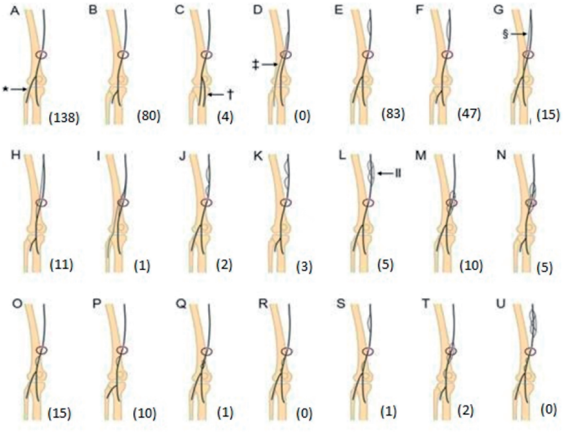


Fig. 3: Variations in deep femoro-popliteal veins (adopted from Park et al, 2011) (4)

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ORIGINAL ARTICLE

Orphan Drugs in Surgery

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Summary
Introduction. Rare diseases, also called orphan diseases, are life-threatening or chronically debilitating conditions of different origin. Majority of them are genetic disorders, others being rare cancers, congenital malformations, autoimmune, toxic and infectious diseases. Rare conditions may also be related to surgery, e.g. acute sensorineural hearing loss after surgery induced acoustic trauma, scarring post glaucoma filtration surgery, and short bowel syndrome following intestinal surgery. Besides, surgery as a specific area for orphan drugs, has not been studied yet in Latvia.
Aim of the study. This study aims to determine orphan drugs associated with surgery (used pre-, during or post-surgery) and their availability and access in Latvia.
Materials and methods. European register of designated orphan medicinal products and EMA approved Summary of Product Characteristics were analyzed, to find orphan drugs with approved labeled indications related to surgery. Drug availability and access in Latvia were determined, by using data available from State Agency of Medicines of Latvia and National Health Service. A literature review was performed to compare Latvian situation in field of orphan medicines with other European countries.
Results. 15 orphan drugs were identified, 8 of them (53.3%) indicated for different kinds of tumors. 6 drugs (40%) are available in Latvia, including one drug (6.7%) included in the reimbursement list.
Conclusions. Oncology is the biggest therapeutic area of orphan drugs. Majority of orphan drugs are not available in Latvia, moreover those drugs that are available are often not accessible.
Key words: orphan drugs; rare diseases; surger; Latvia.

INTRODUCTION

Rare diseases, also related to as orphan diseases, are life-threatening or chronically debilitating conditions of different origin. The majority of them are genetic disorders, others being rare cancers, congenital malformations, autoimmune, toxic and infectious diseases. These rare conditions may also be related to surgery, e.g. acute sensorineural hearing loss after surgery induced acoustic trauma, scarring post glaucoma filtration surgery, and short bowel syndrome following intestinal surgery. Relatively often these conditions are associated with transplantation, for example: ischemia/reperfusion injury associated with solid organ transplantation and recurrent hepatitis C virus induced liver disease in liver transplant recipients. (20) Disease is considered as rare if it affects not more than 1 in 2 000 people in the European Union (EU). It is estimated that between 5 000 and 8 000 different rare conditions exist, affecting 6-8% of the population, concluding that about 30 million people are suffering from rare diseases in the EU. (4)

The EU offers a range of incentives to foster the development of orphan drugs since, under normal market conditions, pharmaceutical companies have little interest in developing drugs intended for small numbers of patients. These incentives include assistance with medicine development, reduced fees for marketing authorization, protection from market competition once the medicine is authorized (10 years of marketing exclusivity). (21) Orphan designation refers to awarding of orphan status to a medicine, but marketing authorization refers to the approval to market the medicine. While many drugs may have received an orphan designation, few have received a marketing authorization. As of March 2013 there are 921 positive opinions on orphan designation and 65 orphan drugs authorized in the EU. Four drugs were withdrawn from use in the EU and six drugs have already completed their period of market exclusivity. (13, 20)

There is a limited public awareness of the rare diseases in general. The national healthcare services for rare diseases differ significantly among the EU Member States, resulting in unequal access to diagnostics and treatment (including orphan drugs). Considering this European Council recommended Member States to establish and implement national plans for rare diseases by the end of 2013. (5) Currently there is no approved national plan for rare diseases in Latvia. A working group for the plan development was established in Ministry of Health in 2010. The plan project called "National Plan for Rare Diseases in Latvia in 2012-2015" was developed by the group and submitted for further public discussion. (15)

Whereas decisions surrounding orphan designation and marketing authorization of orphan drugs are taken at the EU level, decisions governing pricing and reimbursement of orphan drugs are a member state responsibility. Drug reimbursement covers drugs which are included in the Latvian national reimbursement drug list, or based on the medical council's decision, drugs can also be reimbursed within the framework

of individual reimbursement system with limit of 10 000 LVL (€14 229) per patient per year. (15) The main principle of drug inclusion in the reimbursement list is that drug should be therapeutically and cost effective, i.e. decision is value based and is not specific to orphan drugs. The national reimbursement list consists of 3 parts: list A covering therapeutically equivalent drugs; list B that consists of drugs without therapeutic equivalent; and list C that contains drugs for which the annual cost exceeds 3 000 LVL (€4 269) per patient and the manufacturer is obliged to cover treatment expenses for a certain number of patients with his own resources (not less than 10%). The National Health Service evaluates therapeutic value, price, expected budget impact and cost-effectiveness for each drug before it is included in the reimbursement list. Drug price is compared with prices in other EU countries. The price of reimbursed medicine should not be higher than the third lowest price in the Czech Republic, Denmark, Romania, Slovakia and Hungary, and shall not exceed the price of medicine in Estonia and Lithuania. (16)

Currently 29 orphan drugs are available on Latvian market, including 4 drugs that were originally designated orphan medicines, but further withdrawn from the EU register of designated orphan medicinal products upon request of the sponsor (eltrombopag, everolimus, imatinib, and sunitinib). (9) Three drugs are included in the reimbursement list C (imatinib, dasatinib, and nilotinib) all indicated for Philadelphia chromosome positive chronic myeloid leukemia. (22) 13 drugs were reimbursed within the framework of individual reimbursement system in 2008-2012, and four drugs (betaine, idursulfase, mecasermin, and sapropterin) were provided within the program of medicinal treatment of rare diseases in children. Orphan drugs are distributed by both hospital and community pharmacies in Latvia.

All drugs must be authorized before they can be marketed in the EU. However, a first level of accessibility exists for orphan drugs that have not yet been authorized, the most common being compassionate use. It covers diseases for which no satisfactory alternative therapy exists. State Agency of Medicines has approved several programs for drug compassionate use in Latvian hospitals. The programs include influenza medicines (oseltamivir and zanamivir) for intravenous administration, medicine for chronic hepatitis C (boceprevir), and drugs for cancer (dasatinib and pazopanib) used in chronic Philadelphia positive leukemia and metastatic soft tissue sarcoma. Dasatinib (Sprycel) is an orphan drug included in the national reimbursement list C, while pazopanib (Votrient) was originally designated an orphan medicine, but it was further withdrawn from the EU register of designated orphan medicinal products upon request of the sponsor. (3)

AIM OF THE STUDY

This study aims to determine orphan drugs associated with surgery (used pre-, during or post-surgery) and their availability and access in Latvia.

MATERIAL AND METHODS

European register of designated orphan medicinal products (http://ec.europa.eu/health) was used to identify orphan drugs in Europe with European orphan designation and European marketing authorization. For all authorized orphan drugs Summary of Product Characteristics (SPC) approved by European Medicines Agency (http://www.ema.europa.eu) were analyzed to find drugs with approved labeled indications related to surgery (used pre-, during or post-surgery). These indications are not necessarily orphan designated indications of orphan drugs with multiple labeled indications.

For orphan drugs that fulfilled inclusion criteria availability on Latvian market was determined by using National Register of Human Medicines maintained by State Agency of Medicines of Latvia (http://www.zva.gov.lv), as well as directly contacting drug manufacturers and wholesalers. The National Health Service (http://www.vmnvd.gov.lv) data were used to assess drug reimbursement including national reimbursement list and individual reimbursement data in 2008-2012. A literature review was performed to compare Latvian situation in field of orphan medicines with other European countries.

RESULTS

A total of 15 orphan drugs were identified that are used pre-, during or post-surgery (Table 1). 8 drugs (53.3%) are used against different kinds of tumors, 4 of them (26.7%) being used in stem or progenitor cell transplantation. As well dexrazoxane is used for treatment of anthracycline extravasation (an antidote to anthracyclines, which are widely used anticancer medicines) making oncology even wider area for orphan drugs.

Romiplostim and eltrombopag both are used for idiopathic thrombocytopenic purpura, although eltrombopag (as well as imatinib) was originally designated an orphan medicine, it was withdrawn from the EU register of designated orphan medicinal products upon request of the sponsor. A concentrate of proteolytic enzymes enriched in bromelain (NexoBrid) is used to reduce the need and extent of surgical removal of burnt tissue and/or skin transplantation. Wound area left with eschar may require further removal by surgery. According to European Medicines Agency requirements, distribution of NexoBrid should be controlled to ensure that the product is not available for use at a centre, until at least one surgeon at the centre has received formal training in the use of product.

Marketing Authorization Holder for Onsenal has not been able to provide the additional data required to fulfill its specific obligation, as a result of slow enrolment in an ongoing clinical trial for familial adenomatous polyposis (FAP), therefore the product was voluntarily withdrawn from use in the EU in March 2011. However celecoxib is a non-steroidal anti-inflammatory drug (NSAID), marketed by the same manufacturer and in same pharmaceutical form (hard capsules) under the trade

name Celebrex, for symptomatic relief in the treatment of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis and thus it can potentially be used off-label. Another NSAID that is very widely available over the counter is ibuprofen, which is a designated orphan medicine named Pedea for patent ductus arteriosus treatment in preterm newborn infants. While ibuprofen is mainly used as tablet or oral suspension, Pedea is marketed as solution for injection. A course of therapy is defined as three intravenous injections given in 24 hour intervals. The first injection should be given after the first 6 hours of life. If the ductus arteriosus does not close 48 hours after the last injection or if it re-opens, a second course of three doses may be given. If the condition is unchanged after the second course of therapy, surgery of the patent ductus arteriosus may then be necessary.

In case of ziconotide studies the etiologies of pain were varied and included spinal pain, mostly due to failed back surgery. While the overall purpose of dexrazoxane trials was to investigate the efficacy of intravenously administered drug in preventing tissue damage from accidentally extravasated anthracycline, and thus preventing the patients from undergoing the routinely used surgical excision of the affected tissue. A blockbuster anticancer drug imatinib (Glivec) is presented in the current study, since one of indications is adjuvant treatment of patients who are at significant risk of relapse following resection of Kit (CD117) positive gastrointestinal stromal tumors. Although originally it was designated an orphan medicine for wide range of oncological conditions: treatment of chronic myeloid leukemia, malignant gastrointestinal stromal tumors, dermatofibrosarcoma protuberans, acute lymphoblastic leukemia, chronic eosinophilic leukemia and the hypereosinophilic syndrome, and myelodysplastic/ myeloproliferative diseases.

6 out of 15 orphan drugs (40%) included in the analysis are available on Latvian market (Table 2). However availability of drugs does not mean that they are really affordable, making drug reimbursement an important issue. Only one (6.7%) orphan drug, imatinib, is included in the reimbursement list C. Drugs included in the list are reimbursed for a particular indication, but not for all labeled indications. For imatinib the reimbursed conditions are chronic myeloid leukemia and bone marrow transplantation. All other drugs can be reimbursed within the framework of individual reimbursement system. Although in 2008-2012 only 3 drugs were reimbursed though this mechanism: romiplostim, eltrombopag, and plerixafor. There are special requirements for use of Gliolan. It should only be used by experienced neurosurgeons who have completed a training course in fluorescence-guided surgery (fluorescence microscope is used in the procedure) in malignant glioma resection. The Marketing Authorization Holder (MAH) is obligated to implement mentioned training course. According to information provided by MAH there is one neurosurgeon in Latvia experienced in utilizing the product.

The National Health Service also maintains a list of drugs used in hospitals, which are needed for inpatient health care services funded by the state. No orphan drugs included in the analysis were found in the list. If a hospital requires a broader range or some specific products to provide services, an additional, hospital specific list of medicines should be maintained. In current economic situation covering drugs for rare diseases from the hospital budget is doubtful, taking into account high prices of orphan drugs. As an exception Children Clinical University Hospital may be mentioned, as it manages a program of medicinal treatment of rare diseases in children and additional budget resources are available for this program.

DISCUSSION

Our study indicates that majority of orphan drugs associated with surgery are used in oncology field. As well the only drug included in the reimbursement list (imatinib) is indicated for treatment of different kinds of cancer. However this finding is not specific for orphan drugs related to surgery, since survey conducted by the European Organization for Rare Diseases (Eurordis) in 2010 found that rare oncological conditions represented 38% of authorized orphan medicines and 56% of patients potentially treated with these medicines. (12) Similar results were reported by Schey et al. stating that within the total budget impact 40% of the conditions, for which orphan drugs were marketed, were oncological and hematological diseases, accounted for 57% of the total costs in 2010. (23) Thus oncology is the biggest therapeutic area for orphan drugs as entire group. Although the range of orphan indications is dynamic and it is continuously enlarging its field covered by orphan drugs. Whereas historically orphan indications were focused mostly on congenital, metabolic, oncologic and hematologic diseases, now there is a tendency showing that new indications recognized in medical society appear including those associated with surgery, that are covered by orphan definition. For example, medicines for treatment of complications consequencing organ transplantation, and cardiac surgery. A range of surgical indications could be considerably changed in future by the advanced therapies and cell therapies studied recently.

Another finding is that majority of orphan drugs included in the analysis are not available on Latvian market, and only one of studied drugs (imatinib) is included in the reimbursement list. Moreover imatinib is accessible only partially since it is reimbursed for two conditions, while it has six designated orphan indications in the EU. As stated by Drummond et al., because of the small market, orphan drugs are often very expensive. With standard economic evaluation, these drugs usually do not prove to be cost-effective and it, taking into account their high price, means that patient access may be limited. (10) According to Picavet et al. orphan designated drugs have higher median price (€138.56) than non-designated drugs (€16.55) for rare disease indications. (19) Moreover price of an orphan drug is higher for a

disease with a lower prevalence. (6) Although orphan drugs with an alternative have lower annual cost per patient than those without an alternative. (24)

Surveys on orphan drug availability in Europe had pointed out unacceptable delays and inequalities in rare disease patients' access to their medicines. Especially countries with a small population suffer from a longer delay in availability of drugs. (1) Thus in 2010, the number of patients with potential access to orphan drugs ranged from 34% in Greece up to 98% in France. The price also varied between countries, and in some countries it was up to 160% higher than the lowest European price. (12) Another European study found that differences in annual costs per patient between EU countries for a given orphan drug may reach 70%. (6) Denis et al. compared rare disease and orphan drug markets in six European countries. The situation on orphan drug reimbursement in studied countries was as follows: 32 orphan drugs were reimbursed in Belgium (2009); 35 in France (2007); 21 in Italy (2007); 32 in The Netherlands (2009); 28 in Sweden (2008); and 12 in Scotland (2008). (7)

Newer EU Member States are often facing budget restrictions with healthcare budgets much lower than compared to older Member States, thereby reimbursement levels can differ. (14) Thus number of available (marketed) orphan drugs in Bulgaria was 22 and 16 of them were accessible (reimbursed) for patients in 2011. Iskrov et al. point out that this is an important issue especially for Eastern European countries, as a big part of orphan drugs are not priced and reimbursed in many countries. In this geographical and economical region the price level of orphan drugs is not among the lowest in the EU, and that could be explained by the small market size represented by these countries. (11) Serbia might be mentioned as another example, where only four orphan medicines were reimbursed. Authors also suggest that gross domestic product (GDP) value may partly explain differences in the level of orphan drug reimbursement among European countries, since Serbia is a country with a low GDP. (18) In Lithuania budget assigned for reimbursement of orphan medicines is limited and insufficient (6.5 million LTL, i.e. €1.89 million, in 2006), therefore access to health care services and orphan drugs in some cases is restricted. (25)

Currently 29 orphan drugs are available in Latvia, and only three of them are included in the reimbursement list C (imatinib, dasatinib, and nilotinib) all indicated for Philadelphia chromosome positive chronic myeloid leukemia. Thus number of reimbursed orphan drugs in Latvia is smallest among the all European countries included in the analysis. Whereas orphan drugs can also be reimbursed individually with limit of 10 000 LVL (€14 229) per patient per year, this limit is certainly not sufficient. All the other orphan medicines, that are not reimbursed, are practically inaccessible for Latvian patients because of their high costs.

Pharmaceutical companies have to comply with different pricing and reimbursement approaches in each EU country, thereby raising the price of orphan drugs. (2) Moreover prices of drugs distributed through the hospital pharmacies are not regulated in most European countries, but are negotiated directly between the manufacturer and the hospital. According to Simoens, there is a need for a transparent and evidence based approach towards pricing and reimbursement of orphan drugs. (24)

The economic impact of orphan drugs on national budget is growing, for example, in France representing a total budget of €1 billion in 2009. (14) The annual per patient cost of orphan drugs varied between €1 251 and €407 631, with the median cost being €32 242. The share of the total European pharmaceutical market represented by orphan drugs was 3.3% in 2010, and it was predicted by Schey et al. to increase to a peak of 4.6% in 2016. (23) Another analysis estimated that orphan drugs constituted 1.9% of total drugs expenditure in Belgium in 2008, and predicted it to increase to about 4% in 2013. (8) While the average budget impact of orphan drugs accounted for 1.7% of the total pharmaceutical expenditure across France, Germany, Italy, Spain and the UK in 2007. (17)

CONCLUSIONS

Oncology is the biggest therapeutic area of orphan drugs. However it is specific for orphan drugs as entire group, rather than for orphan drugs that are exclusively related to surgery.

Majority of orphan drugs are not available in Latvia, moreover those drugs that are available are often not accessible because they are insufficiently reimbursed by the state, and are too expensive to be covered by patients.

Conflict of interest: None

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Table 1. Orphan drugs associated with surgery

Active substance	Trade name	Approved labeled Indication
Concentrate of proteolytic enzymes enriched in bromelain	NexoBrid	Removal of eschar in patients with deep partial- and full-thickness thermal burns
Romiplostim	Nplate	Treatment of chronic idiopathic thrombocytopenic purpura (ITP) in splenectomised patients who are refractory to other treatments (corticosteroids, immunoglobulins)
Eltrombopag	Revolade	
Teduglutide	Revestive	Treatment of short bowel syndrome. Patients should be stable following a period of intestinal adaptation after surgery
Ibuprofen	Pedea	Treatment of a hemodynamically significant patent ductus arteriosus in preterm newborn infants
Ziconotide (intraspinal use)	Prialt	Treatment of severe, chronic pain in patients who require intrathecal analgesia
Dexrazoxane	Savene	Treatment of anthracycline extravasation
Celecoxib	Onsenal	Reduction of the number of adenomatous intestinal polyps in familial adenomatous polyposis (FAP), as an adjunct to surgery and further endoscopic surveillance
5-aminolevulinic acid hydrochloride	Gliolan	Visualization of malignant tissue during surgery for malignant glioma
Mifamurtide	Mepact	Treatment of high-grade resectable non-metastatic osteosarcoma in children, adolescents and young adults after macroscopically complete surgical resection
Imatinib	Glivec	Adjuvant treatment of patients who are at significant risk of relapse following resection of Kit (CD117)-positive gastrointestinal stromal tumors (GIST)
Brentuximab vedotin	Adcetris	Treatment of relapsed or refractory CD30+ Hodgkin lymphoma following autologous stem cell transplant
Busulfan (intravenous use)	Busilvex	Conditioning treatment prior to conventional hematopoietic progenitor cell transplantation (HPCT)
Thiotepa	Tepadina	Conditioning treatment prior to allogeneic or autologous HPCT in hematological diseases; when high dose chemotherapy with HPCT support is appropriate for the treatment of solid tumors
Plerixafor	Mozobil	Treatment to mobilize hematopoietic stem cells for subsequent autologous transplantation in patients with lymphoma and multiple myeloma

Table 2. Surgery related orphan drugs available in Latvia

Active substance	Trade name	Reimbursement category	Reimbursement conditions
Imatinib	Glivec	List C	Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML); bone marrow transplantation
Romiplostim	Nplate	Individual	Has been reimbursed for essential (hemorrhagic) thrombocythemia
Eltrombopag	Revolade		Has been reimbursed for neoplasms of uncertain or unknown behavior of lymphoid, hematopoietic and related tissue
Plerixafor	Mozobil		Has been reimbursed for nodular sclerosis and follicular lymphoma
5-aminolevulinic acid hydrochloride	Gliolan	Individual	These drugs are available in Latvia, but are neither included in the reimbursement list, nor reimbursed individually in 2008-2012
Ibuprofen	Pedea		

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PROBLEM-SOLVING ARTICLE

Renal Cell Carcinoma – How Can We Predict its Outcomes in Clinical Practice?

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Summary
Morbidity and mortality data of RCC (renal cell carcinoma) differs a lot among the European countries. In Latvia a growing trend in both incidence and mortality rates is still observed. The expanding availability of multiple treatment strategies has increased the importance of skilled individualized outcome prediction for patients. Several prognostic factors are available in RCC including anatomical, histological, clinical and molecular ones, but none of them is very precise, when used alone. Therefore increasing number of prognostic systems has been created in local and metastatic disease to increase predictive accuracy. In order to encourage the clinicians to use the available models in their routine practice, we tried to select the most relevant ones and include them in a simple algorithm to be used in common clinical scenarios throughout entire history of the disease in patients with RCC.
Key words: renal cell carcinoma; prognostic factors; prognostic models.

INTRODUCTION

RCC accounts for approximately 90% of all kidney malignancies and represents 2–3% of all cancers, with the highest incidence in Western countries (28). During the last two decades an annual increase of about 2% in incidence is observed both worldwide and in Europe, however in some countries, as Denmark and Sweden decrease is reported. In Europe, overall mortality rates for RCC have increased until the early 1990s, but thereafter the situation has very much changed in some European countries and less in others. Decrease in mortality rates is observed in Scandinavian countries already since the 1980s, but in Austria and Netherlands, since the early 1990s. However, in some European countries, including Latvia, mortality rates still show an upward trend (10).

Management of metastatic RCC has significantly changed in the last few years with the development of targeted therapies. At present, several antiangiogenic drugs: tyrosine kinase inhibitors, monoclonal antibodies have been approved both in the USA and in Europe for the treatment of metastatic RCC. Significant differences in mortality rates among the European countries could be explained by the limited availability of the new treatments. At present, in Latvia none of the new medications is completely reimbursed and just a small number of patients have been on the targeted treatment within investigational protocols or individual compensation request. Taking into consideration the growing treatment costs and limited efficacy, that has to be balanced with the risk of side effects, physicians involved in the treatment of RCC, especially in the countries like Latvia with limited financial resources, increasingly need accurate tools in prediction of treatment response in order to select the patients who may benefit most.

RCC – DIFFICULT TO PREDICT AND TO MANAGE

RCC is a heterogeneous and complex disease with variable prognosis. Treatment decision and creation of appropriate follow–up plan should be based on prediction of response to therapy and on the prognosis of recurrence and survival. Unfortunately in case of RCC there is no specific marker to help in monitoring the disease and estimating treatment efficacy. Several non–specific factors can be used to predict the disease outcomes, however none of them is perfectly accurate when used alone. Therefore a number of prognostic models in the form of scoring algorithms or nomograms combining different prognostic variables have been developed in order to improve predictive accuracy.

At present TNM stage is the most important prognostic tool in RCC, and most likely will remain so in the future. Patients with stage pT1 or pT2 (organ confined) disease have the best prognosis, with 5–year cancer–specific survival rate after nephrectomy ranging from 67% to 94%; for patients with locally advanced tumors it decreases by 23% to 67%, and once RCC has metastasized, it is less than 23% (45). However in real life individual patient cases show significant differences in tumor behavior within all stages, resulting in variable survival prognosis.

Research continues to detect strong and easily available prognostic parameters that may help to classify patients into groups with different risks for death from renal cancer. As a result in the last 15 years a number of prognostic systems combining several independent factors have been developed to improve the predictive accuracy provided just by TNM stage. An important advantage of these models, based on mathematical calculations and statistical estimates, is their ability to measure the predictive accuracy, which enables all new variables to be objectively evaluated. In the world of extremely rapid flow of information, we tried to compile

the data published so far, by selecting the most important prognostic factors and their integrated systems in order to make them more applicable in everyday clinical use. A literature review was performed using the PubMed database for articles published by January 1, 2013. As the first step we examined the systematic reviews and meta–analysis, followed by the analyses of each study data.

Classical prognostic factors for RCC can be divided into anatomical, histological, clinical and molecular, which differ in local and metastatic disease (43). We tried to identify the ones that can be easily detected in routine practice by the clinicians.

PROGNOSTIC FACTORS IN NON–METASTATIC RCC

Anatomical factors are the ones, that are integrated in the TNM staging system (tumor node metastasis) and include: tumor size, growth beyond renal capsule into fat or peri–sinus tissues, invasion of renal vein and/or inferior vena cava, adrenal extension and lymph node invasion. The TNM classification undergoes continuous improvements, each version, including the last 2009 has introduced significant changes based on recent studies, because of their prognostic relevance (28). As an example: in previous TNM classifications, the pT3b group included both renal vein and inferior vena cava invasions, that have been separated in the latest version of the TNM classification; adrenal invasion has a poor prognosis, so it has been re–classified as pT4 tumor.

There are still some unresolved issues regarding TNM staging. One of them is related to nodal invasion, which has been confirmed to be an independent prognostic factor regardless of T stage. However, the necessity of the sub–classification of nodal involvement according to the number of affected nodes (N1: 1 node involved; N2: > 1 node involved) is not clear. Furthermore, in the 2002 TNM classification it is recommended that at least 8 nodes should be removed for nodal staging. Below this number of nodes, the patient should be staged as Nx. In clinical practice, this number of removed nodes is not always achieved, especially in case of partial or laparoscopic nephrectomy (28,42, 43).

Another issue applies to renal sinus fat invasion, which has been classified as pT3a since the 2002 version of the TNM classification. However, data suggest that renal sinus fat invasion carries a worse prognosis than perinephric fat invasion and therefore should not be included in the same stage group (1).

Histological factors. According to the World Health Organization (WHO) there are 3 major histological subtypes of RCC: clear cell, papillary and homophobe (28). At least few studies are proving significant correlation between histologic subtype and disease specific survival in univariate analysis, with clear cell RCC being the most aggressive tumor followed by papillary and homophobe. Papillary tumors are divided into two groups with very different prognosis: type I and II. However, the prognostic value reduces in multivariate analysis, suggesting that stage and grade have a higher impact on prognosis than histologic subtype. Histological features of the highest prognostic value include: Fuhrman nuclear grade, presence of sarcomatoid differentiation, micro–vascular invasion, necrosis, collecting system invasion (2,30).

Clinical factors. The classic triad of flank pain, gross hematuria, and palpable abdominal mass is now rare (6–10%) (9,43). The presence or absence of classical symptoms mentioned above, along with constitutional symptoms has shown o have some prognostic significance in local disease. Performance status (PS) assessed by the ECOG or Karnofsky has been found to have significant association with prognosis in several studies. It has been proved by the study at University of Michigan that the way of presentation of the disease symptomatic vs. incidental may also serve as an independent prognostic factor for survival (26).

Molecular Factors. Numerous molecular markers have been studied for the use in the management, prognosis and follow up of local RCC. However, at present the available data doesn't support routine clinical application of any. Panels of several markers are likely to be of higher predictive value than a single one. Further research is needed to assess the utility of biomarkers in the clinical work–up of patients with RCC (11).

PROGNOSTIC FACTORS IN METASTATIC RCC

Anatomical factors. In the metastatic setting, the classical anatomical factors (stage, size, venous, adrenal invasion) that have been previously described for local RCC, have limited prognostic role. It is generally considered that the prognostic impact of primary tumor variables decreases as soon the tumor spreads and becomes metastatic. Anatomical site of metastases has some prognostic role, but resectability of metastases is even more relevant independent prognosticator regardless of the site. In case of lung metastases, the presence of multiple lesions and associated lymph node involvement correlates with worse outcomes. Patients with greater than one site of metastases, are associated with poorer prognosis (20).

Histological factors. The histological features of possible prognostic value in metastatic RCC are histological subtype and the presence of sarcomatoid component. Taking into account that non–clear cell tumors are worse responders to cytokines, this has been considered an important factor in the era of immunotherapy. At present, when the antiangenic drugs are becoming the first treatment choice, the predictive relevance of histological subtype needs to be clarified. The presence of sarcomatoid differentiation in metastatic RCC is clearly related to worse prognosis (43).

Clinical factors. Clinical picture of the disease becomes of special importance once the tumor develops metastases. There are 4 groups of factors (40):

1) Patient factors include constitutional symptoms such as weight loss, decreased appetite, musculoskeletal pain, sweats, respiratory and gastrointestinal symptoms, having negative impact on survival (40). PS is the most important clinical prognosticator in metastatic RCC. This has been clearly established both in the immunotherapy and targeted therapy era (5).

2) Tumor burden markers include elevated lactate dehydrogenase (LDH) due to high cell turnover, hypercalcemia due to bone metastases or production of parathyroid hormone related peptide, hyponatremia due to syndrome of inappropriate antidiuretic hormone secretion and anemia (19,21,14).
3) Proinflammatory markers. With the development in cancer research, it has become clear that cancer progression depends on a complex interaction between the tumor and the host inflammatory response. Clinically the systemic inflammatory response can be evaluated by a number of markers: elevated C–reactive protein (CRP) levels, accelerated erythrocyte sedimentation rate (ESR), or increased white cell, neutrophil and platelet counts. Among the inflammatory indicators CRP is reported to be superior to other inflammatory markers. The recent meta–analysis published by Wu et al. compiling 47 studies' data, suggests that CRP, thrombocyte count and ESR are essential factors for predicting tumor–specific survival with estimated hazard ratios (HR) accordingly 3.46, 3.22 and 3.85 (44).
4) Treatment–related factors include presence or absence of previous nephrectomy and duration of disease free interval (DFI). The interval between nephrectomy and development of metastatic disease is inversely linked to prognosis (7). For patients who present with metastatic disease and good PS cytoreductive nephrectomy prior to imunotherapy improves survival if compared to interferon–a alone, that has been proved by randomized studies (12).
Molecular factors. A large number of genes and proteins have been tested as potential prognostic factors for metastatic RCC. Since targeted therapies are directed to well–defined molecular targets, there is a strong rationale to assess these targets also as prognostic markers. For example, the VEGF gene family, CAIX, VEGFRs, PDGFRs, VHL status are good candidates to analyze the VHL pathway in clear cell RCC, but pAkt, PTEN, p27, and pS6 can be useful for exploring the mTOR pathway in clear cell and non–clear cell RCC. However, no study has clearly established yet the usefulness of one or more of these factors in the routine clinical practice (17,11).

PROGNOSTIC SYSTEMS

Most of the features mentioned above, in particular TNM stage, Fuhrman nuclear grade and PS can be used as independent prognostic factors. However to achieve higher accuracy in outcome prediction the use of integrated systems that combine multiple independent prognostic variables is recommended. Models proposed by various authors differ by analyzed variables, selected patient population, predicted outcomes and prognostication methods: stratification into risk groups or individual risk assessment. However, not all of them are appropriate for daily clinical use and decision making, because of following drawbacks:

1) do not provide individual level predictions;
2) are weighted towards limited number of patients;
3) no external validation;
4) applicable for selected patient population with definitive histology, stage or previous treatments.

All these criteria have to be considered, when selecting the most appropriate model in the particular clinical scenario, which complicates and limits their routine use outside investigational projects. In this article, we summarized the currently proposed models for prediction of outcomes for both surgical and pharmacological treatments in RCC. The aim of this overview is to encourage the clinicians in using the suggested scoring algorithms and nomograms for decision making in specific clinical situations. Variables used in the models are not detailed as most of them are detected in routine clinical setting. At the time this article is created there at least several comprehensive reviews available (11,17,26,38,40,43) and we will not go into thorough description of each system, but will try to emphasize the most useful ones, by selecting the models of most clinical utility in assisting physicians in their intense work.
The ideal prognostic model should meet the following criteria (13,15):

1) should be calibrated and externally validated in the independent patient group producing high accuracy;
2) should reflect the situation not just in one center, but in wider population;
3) should meet the contemporary requirements, when new diagnostic and therapeutic techniques are available comparing with the historical patient cohort;
4) should provide individual risk evaluation of each patient;
5) should be simple and easy to use;
6) should not include costly or complicated tests, which cannot to used in clinical practice or are inaccessible on daily basis.

Unfortunately, in general, prognostic accuracy of the models recommended for metastatic disease is significantly worse as for local RCC. One of the possible reasons for that is that information on the primary renal tumor is excluded e.g., histological factors and status of the lymph nodes. It can be partially explained by the lack cytoreductive operations in historical series in metastatic renal tumors. Theoretically, it is possible that inclusion of the primary tumor characteristics could improve the prognostic accuracy; however, today more attention is paid to molecular markers. It is therefore expected that further improvements will be related to inclusion of biological and genetic features in the currently approved systems (26,38).
By reviewing systematic overviews and separate study articles, totally 46 prognostic systems proposed by various authors were identified. When comparing published data, the most established and precise models where selected. To make practical application more convenient the proposed models are grouped according to the purpose they can be used for.

1) Due to the wider availability of imaging techniques, the number of patients with small, asymptomatic renal masses has increased. Excision is the usual standard care in such cases, although only 10–30% of them are potentially aggressive. Several

prenephrectomy models are created to predict the presence of RCC, possible metastatic progression and RCC–specific mortality. Over the past decade, at least 9 models predicting different endpoints prior to nephrectomy have been developed (17). We would like to mention here model created by Kutikov et al. that allows selection of patients that are at higher risk of death from RCC as from other causes (23). In low–risk patients close monitoring of the disease or less aggressive ablation treatments could be offered. The nephrometry scale proposed by the same author is another useful tool in evaluating the potential of tumor malignancy prior operation (24).

2) UCLA Integrated Staging System (UISS) and the Mayo Clinic's SSIGN score for many years have been the two most used prognostic models for localized RCC (45,13). Several new predictive systems for estimating survival outcomes after nephrectomy for patients with localized RCC are developed in the past decade. There have been attempts of doing direct comparison between the proposed models, however the obtained data is not convincing in favor to one certain model. Currently there are several ongoing adjuvant treatment trials in high–risk patients after nephrectomy in which different models for risk estimation are selected. More evidence is expected after their completion (15). In 2009 Karakiewicz et al. proposed a new nomogram for prognosis of RCC, in which tumor size is used as a continuous variable and the ECOG performance status is replaced by symptom classification, which is likely to be more acceptable to urologists than PS (18). Another beneficial feature of this model is its abiliy to provide individual estimation of RCC–specific survival instead of grouping patients into risk categories. Furthermore, the multi–institutional data set makes this model more likely to be applicable for patients treated with nephrectomy at other centers. The Karakiewicz model is easy to use (online version is available), has high predictive accuracy, which makes it attractive for individual patient counseling. A recent study by Tan et al. has demonstrated that the postoperative Karakiewicz nomogram achieves superior survival prediction providing higher clinical benefit, comparing to other tested models (39).
3) For prediction of recurrence in local RCC after nephrectomy the system created in 2005 by Sorbellini et al. could be helpful (37). This model achieved 82% accuracy in external validation, but is applicable only to clear cell RCC.
4) Metastatic RCC has a very poor prognosis with 5–year survival not exceeding 20% (45). However the natural course of the disease in these patients may differ a lot. Several prognostic models have been proposed, but only few have been assessed for their predictive accuracy. The French group of immunotherapy (35) and different versions of Motzer's models (33,32) from the Memorial Sloan–Kettering center (MSKCC) are the two prognostic tools, which have been widely adapted in the clinical practice. 5 prognostic factors that have been identified by multivariate analysis including low Karnofsky PS, elevated LDH, low serum hemoglobin, high corrected serum Ca, absence of nephrectomy or time from diagnosis to initiation of systemic therapy, depending on the version, are used for patient stratification in the Motzer's models. Despite its acceptance by clinicians, it has few drawbacks, including no conclusive predictive accuracy, lack of the variables for the primary tumor and no consideration of lymph node status. A slightly modified version of the Motzer's criteria have been tested by Escudier et al. adding other two variables: alkaline phosphatase (AP) and number of metastatic sites (8). Another model has been proposed by Leibovich et al., along with the prognostic value, the importance of which is related to a subgroup of 192 patients in whom the metastatic RCCs were completely resected, that resulted in approximately 60% survival at 3 years after surgery (27). This finding emphasizes the need for surgical treatment in feasible patients at least by the time systemic therapies for metastatic RCC do not prove a curative effect.

   Two other systems developed recently and being of potential interest are the one published by Iimura et al. (16) with reported external validation score of 86%, and the second one by Manola et al. (29) including data of 3 748 patients in clinical trials with separate data set from patients on tyrosine kinase inhibitors. 9 prognostic factors were identified for survival in metastatic disease: PS, number of metastatic sites, time from diagnosis to treatment, pretreatment hemoglobin, white blood cell count, LDH, AP and serum calcium.
5) The current selection of targeted agents has been mostly based on clinical efficacy, side effect profile, comorbidities and PS. However, with a rapid expansion of targeted drugs, choosing the most appropriate one is becoming increasingly difficult. That's why recently the significance of prognostic systems in metastatic setting has particularly increased in prediction of the response to systemic treatment. Motzer et al (34). reported the first nomogram to predict 12 months progression–free survival after first–line treatment with sunitinib. Another prognostic system was proposed by Choueiri et al. to determine survival after antiangiogenic therapy (3). A multivariate analysis of risk factors adversely associated with PFS identified an ECOG PS score $\geq$1, time from diagnosis to treatment <2 years, and corrected serum calcium level >10 mg/dl. Two additional risk factors that have been identified in previous studies, high platelet count (>300 K/mcl) and high absolute neutrophil count (>4.5 K/mcl), were of some significance as well. Similar model has been applied by Heng et al. by analyzing a cohort of 645 patients with metastatic RCC (all subtypes) treated with VEGF–targeted agents (14).

At present patient risk stratification proposed by Motzer (33) based on 5 predictive factors as mentioned above is recommended by the guidelines of European Association of Urology (EAU) (28) and ESMO (9) for selection of systemic treatment in metastatic setting and has become an accepted standard in many clinics. According to the National Comprehensive Cancer Network (NCCN) guidelines (31) only poor–prognosis group is distinguished in which temsirolimus is recommended based on the extended 6–factor model provided by Hudes et al., in addition to 5 Motzer's criteria the presence of multiple metastases is also considered (15).

6) For prediction of survival in patients having recurrence after nephrectomy, special model proposed by Eggener et al. based on similar criteria as suggested by R. Motzer, may be used (6).
7) For selection of patients with metastases that could benefit from surgical treatment a special model has been developed by Culp at al. (4). Since the high–risk patients experience significantly lower benefit from cytoreduction, any other algorithm applicable for metastatic RCC can be used for this purpose (26).
8) After surgical excision, 20% to 30% of patients with localized tumors experience relapse. The median time to relapse after surgery is 1 to 2 years, with most recurrences occurring within 3 years (28). At present there is no consensus on surveillance after treatment for RCC. The main reason for control is to identify local recurrence or metastases early. Intensive radiological surveillance for all patients is unnecessary. For example, the outcome after surgery for T1a, low–grade, tumors is almost always excellent. It is therefore reasonable to plan follow–up, taking into account the risk of a recurrence. Scoring systems and nomograms designed by Liebovich, UCLA, and Karakiewicz (27,45,18), can be easily adapted to estimate the likelihood of RCC patients of developing tumor local recurrence or metastases. As recommended by EAU guidelines (28) surveillance after treatment for RCC should be based on a patient's risk group: for low–risk disease, the use of CT can be infrequent; in the intermediate–risk group, an intensified follow–up that includes CT scans at regular time intervals should be performed according to a risk–stratified nomogram; in high–risk patients, the follow–up examinations should include routine CT scans.
According to (NCCN) guidelines (31) no single follow–up plan is appropriate for all patients and it should be individualized based on the patient and tumor characteristics.
Lifelong surveillance is necessary only for some patients with RCC. Late recurrences more than 10 to 20 years after nephrectomy are rare, but single cases have been reported as long as 45 years after initial surgical resection (41). The appropriate intensity of follow up after 5 years remains to be established.
9 Most of the nomograms are designed after 1999 and does not include any of the possible biomarkers. It is expected that the incorporation of molecular factors into standard predictive nomograms may lead to higher prognostic and predictive accuracy. As a result recently several models have been created with integrated molecular information.

Kim et al. screened RCC patients using a tissue microarray technique to examine 29 markers related to the hypoxia–inducible and rapamycin pathways were evaluated (22). A unified nomogram was developed including Ki–67, p53, endothelial VEGFR–1, epithelial VEGFR–1, and epithelial VEGF–D along with ECOG PS, T stage, Fuhrman grade to stratify patients into risk groups. Addition of molecular markers along with classic variables improved the predictive accuracy considerably (concordance index of 0.9). Parker et al. created another biomarker–based scoring system in patients with clear cell RCC called BioScore by integrating information on B7–H1, Ki–67, and survivin. Patients with high BioScores (>4) were 5 times more likely to die from RCC than those with low scores. In addition, the sequential use of BioScore with existing scoring systems (TNM, UISS, SSIGN) enhanced their predictive ability compared to each of these scoring systems alone, which makes it quite attractive for further evaluation in investigational and clinical settings (36). Neither clinical data, nor biological information can be treated in isolation, as both are relevant to patient care and outcomes. It is expected that the future success of biomarker studies will lead to modification of existing systems or developing new ones based on large–scale multivariate analysis.

More experienced professionals when assessing the prognosis and selecting the appropriate treatment in RCC patients use their observations and intuition developed over the years, the youngest colleagues act in accordance to literature studies. However, even if the one is fairly confident in relying just on personal feelings and knowledge, it needs to be considered that more objective methods for prediction and management of this heterogeneous disease are available.

CONCLUSIONS

1) In several European countries, including Latvia, not only morbidity but also mortality rates of RCC continue to increase.
2) Survival of RCC patients depends mainly on a TNM stage; however, among the patients of each stage different course of disease is observed. Certain factors – analyzed separately and in combinations – are essential for prognosis of RCC outcomes and prediction of treatment response.
3) 46 prognostic systems developed by several authors have been identified, having less or more significant differences in the analyzed outcomes and variables, selected patient populations, previous treatments used and purpose of application.
4) The most accurate and commonly used models have been selected according to their intended use, including management of small renal masses,

identification of patients requiring cytoreductive or systemic treatment, guidance of the follow up and advising on disease outcomes. We propose a simple decision making algorithm in common clinical scenarios throughout entire history of the disease in patients with RCC by indicating the possible prognostic systems to be used at each step (Table 1).

Conflict of interest: None

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Table 1. Decision making algorithm for patients with renal cell carcinoma

I Initial investigations

1. H&P: Concomitant diseases and treatments
smoking history, PS (Karnofsky or ECOG)
presence of local symptoms (hematuria, flank mass, pain), severety of constitutional symptoms

2. Laboratory evaluation: FBC, urinalysis, biochemistry: including liver function tests, creatinine, LDH, AP, corrected Ca, CRP

3. Radiological investigations:
Abdominal / pelvic CT (with or without contrast depending on renal function):
Cystic mass: Bosniak classification / Solid mass: Nephrometry scale, Kutikov 2011 (24) To be assessed in all: extension to Gerota's fascia & surrounded tissues; necrosis; extension to veins; status of regional l/n; extension to adrenal gland; distant metastasesIn all – Chest XR; If clinically indicated – chest CT, bone scan, brain MRI.

II Clinical staging, risk evaluation and treatment decision

Stage		**Possible treatment**	**Decision making**		**Stratification tools**
IA		Partial nephrectomy or radical nephrectomy or thermal ablation or active surveillance	1) Tumour factors: e.g., localization 2) Patient factors: e.g., PS, concomitant diseases, age 3) Risk assessment		Kutikov 2010 (23)
IB		Partial or radical nephrectomy			
II & III		Radical nephrectomy	Adjuvant therapy in clinical trials		
IV R E L A P S E	Solitary metastasis	Nephrectomy + metastasectomy	Risk assessment		Culp 2010 (4)
	Potentially resectable primary with multiple metastasis	Cytoreductive nephrectomy prior to systemic therapy	Risk assessment		
	Unresectable	Systemic therapy or palliative care	1) Histology 2) Risk assessment		Motzer 2002 (33)
			Prediction of response to:	cytokines sunitinib sunitinib, sorafenib, bevacizumab temsirolimus	Negrier 2002 (35) Motzer 2008 (34) Heng 2009 (14) Hudes 2007(15)

III Histological examination of specimen and pathological staging
IV Individualized follow up based on risk assessment

Investigations to be done at control visits	**Stratification tools**
1) H & P; 2) Laboratory evaluation: CBC, urinalysis, biochemistry: including liver function tests, creatinine, LDH, AP, corrected Ca, CRP; 3) Radiological investigations: chest XR, abdominal US or abdominal/ pelvic CT	All: Zisman 2001 (45); Karakiewicz 2007 (18) Local: Kattan 2001 (56) Metastatic: Leibovich 2005 (45)

Disease outcome	**Prognostic tools**
Prediction of recurrence in local disease	Sorbellini 2005 (37)
Survival prognosis in local disease	Zisman 2002 (45); Frank 2002 (13); Karakiewicz 2009 (18)
Survival prognosis in metastatic disease	Iimura 2009 (16); Manola 2011 (29)
Predictions of rapid progression (3 months) in metastatic disease	Negrier 2002 (35)
Prognosis of survival after recurrence	Eggener 2006 (6)

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PROBLEM-SOLVING ARTICLE

Hereditary Gastric Cancer: Review of Literature

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Summary
Worldwide, gastric cancer is one of the most common forms of cancer, with a high morbidity and mortality. Both environmental and genetic factors have a role in the aetiology of gastric cancer. Familial clustering of gastric cancer is seen in 10-15% of cases, and approximately 3% of gastric cancer cases arise in the setting of hereditary diffuse gastric cancer (HDGC). In families with HDGC, gastric cancer presents at relatively young age. Germline mutations in the *CDH1* gene are the major cause of HDGC and are identified in approximately 25-40% of families which fulfill strict criteria. Prophylactic gastrectomy is the only option to prevent gastric cancer in individuals with a *CDH1* mutation. However, in the majority of families with multiple cases of gastric cancer no germline genetic abnormality can be identified and therefore preventative measures are not available, except for general lifestyle advice. Future research should focus on identifying new genetic predisposing factors for all types of familial gastric cancer.
Key words: gastric cancer; hereditary diffuse gastric cancer; *CDH1;* E-cadherin; prophylactic gastrectomy.

INTRODUCTION

Gastric cancer (GC) are major health problems in many countries throughout the world. With an estimated 989,000 new cases per year (8.0% of new cancer cases) it is in fourth place behind cancers of the lung, brest, and colon and rectum. Even though the incidence is rapidly declining in the western world, it is still the second most common cause of death from cancer, with 740,000 deaths annually (20). Gastric cancer's incidence shows large geographic differences worldwide. High-risk areas include East Asia (Japan, China and Korea), Eastern Europe, and parts of Central and South America (ASR in men, > 40 per 100,000). Incidence rates are low in North Europe, North America and Australia (< 15 per 100,000) (20).

In Latvia gastric cancer takes the 4th place among the whole population (3rd place in males and 6th place in females) and the prognosis of advanced gastric cancer remains poor. The age-standardized incidence rate of gastric cancer in Latvia is higher than in European Union (EU) – 28.6 cases and 14.6 cases of gastric cancer (per 100 000) were reported in men and women, respectively, in Latvia. The EU age-standardized incidence rate is lower: 18.2 cases and 8.1 cases per 100000 are reported in men and women, respectively. The age standardized mortality rate in Latvia is also at least twice higher than reported in the EU: 27.5 cases and 12.0 cases per 100 000 in men and women, respectively, versus 12.2 cases and 5.7 cases per 100 000 in men and women, respectively, in the EU (20).

Gastric cancer incidence worldwide is more than double in men than in women (rate ratio 2.2:1.0). With a mean age at diagnosis above 60 years, gastric cancer is predominantly a disease of the elderly (36). Only 6–7% of patients with gastric cancer present before the age of 50, and less than 2% before age 40. Gastric cancer is usually diagnosed at an advanced stage, with > 50% of patients having stage 3 or 4 disease at presentation. Advanced gastric cancer has a poor prognosis, with a relative 5- year survival rate of 7-27% (10,36).

Classification

Gastric carcinoma is a heterogeneous disease, which is reflected by the diversity of the various histopathological classification schemes (4). The most commonly used are those of the WHO (4) and Laurén (27). The practical scheme of Laurén divides GC roughly into three main types; the diffuse type, the intestinal type and a rest group composed of mixed and indeterminate type (3). The intestinal type is more common in the general population, more likely to be sporadic and related to environmental factors. Intestinal GC shows glandular or tubular components with various degrees of differentiation. The diffuse type is more likely to have a primary genetic etiology, a subset of which, known as hereditary diffuse gastric cancer (HDGC), is due to the E-cadherin (CDH1) germline mutation. Diffuse GC consists of poorly cohesive single cells without gland formation. Often signet ring cells are present; therefore it is also referred to as signet ring cell carcinoma (26). In North America the distribution of the different subtypes is approximately 50% pure intestinal, 35% pure diffuse and 15% mixed diffuse-intestinal (32).

Etiology of gastric cancer

Gastric cancer is a multifactorial disease, resulting from a combination of environmental factors and genetic alterations. Environmental factors are mainly involved in the etiology of the intestinal type of GC. The main environmental factor involved is *Helicobacter pylori* (*H. Pylori*) infection, smoking and diet. In young people, in whom carcinomas are more likely to be due to genetic susceptibility, a greater proportion shows the diffuse type, suggesting that especially in this subtype germline genetics play a role (4). Familial aggregation of gastric cancer is known to occur in approximately 10-15% of

the patients (26). Epidemiologic studies have shown that in the general population the risk of gastric cancer in first-degree relatives with any type of gastric cancer is increased 2–3 fold (16). As yet, however, in the vast majority of these patients the underlying genetic cause remains unknown. The most important GC susceptibility gene is *CDH1*, which accounts for 1-3% of gastric cancers (35). Predisposing *CDH1* mutations have been encountered in about 30% of strictly selected Hereditary Diffuse Gastric Cancer (HDGC) families (21,31). Moreover, *CDH1* germline mutations may also occur in approximately 7% of patients diagnosed before 50 years of age with tumors exhibiting either a diffuse or a mixed histology (9).

Hereditary diffuse gastric cancer caused by germline *CDH1* mutations

Hereditary diffuse gastric cancer (HDGC) is defined as a syndrome of inherited predisposition to cancer with an autosomal dominant inheritance pattern.

In 1998, Guilford *et al*. identified germline mutations in the *CDH1* gene as a cause of hereditary diffuse gastric cancer (17). *CDH1* encodes the protein E-cadherin, which plays an important role in cell–cell adhesion and the maintenance of epithelial integrity (3). The mutation detection rate is approximately 50% in families with two gastric cancers in first-degree relatives with at least one diffuse gastric cancer (DGC) diagnosed before age 50, or three or more DGC in close relatives diagnosed at any age (31). The percentage decreases if also single cases of DGC below the age of 35 are included (21). Germline *CDH1* mutations are found in all ethnic groups (13).

The most common types of mutation are small insertions or deletions (35% of the mutations). Missense mutations occur in 28% of families, nonsense mutations and splice site mutations are both observed in 16% of families. Large exonic deletions are relatively rare, with a frequency of about 5% (5).

For both men and women, *CDH1* mutation carriers have a cumulative risk of gastric carcinoma by 80 years of age of 80%, with a mean age at diagnosis of 40 years. Additionally, women carrying a *CDH1* mutation have a 60% lifetime risk for developing lobular breast cancer (13).

Genetic counseling and criteria for *CDH1* mutation testing

Genetic counseling is an essential component of the management of HDGC. It includes the analysis of the family history of at least three generations and histopathological confirmation of gastric (pre) malignancies. The revised international criteria as established by the International Gastric Cancer Linkage Consortium (IGCLC) to select patients with an increased risk of familial gastric cancer for *CDH1* mutation testing:

1. diffuse gastric cancer case below age 40, or
2. gastric cancer cases in a family, one confirmed diffuse gasrtric cancer below age 50, or
3. confirmed diffuse gastric cancer cases in 1st or 2nd degree relatives independent of age,or
4. personal or family history of diffuse gastric cancer and lobular breast cancer, with one diagnosis below age 50.

(13). Genetic testing is preferably initiated in an affected relative. In most countries the youngest age at which relatives at risk should be offered testing is set at age 18. Rare cases of gastric cancer before age 18 have been reported, but the overall risk of DGC before the age of 20 is very low (1,33).

Proposed mechanism of HDGC initiation

In 2009, Humar and Guilford proposed a mechanism of HDGC initiation (18). E-cadherin is known to play an important role in cell polarity and epithelial tissue architecture (11,29). It is proposed that mutations in *CDH1* disturb the cell-cell adhesion mediated by E-cadherin, which causes disruption of the correct spatial organization of the cells. This in turn may interfere with processes that regulate cell division, such as the orientation of the mitotic spindle. Abrogated cell polarity may also lead to the disruption of cell fate determination (18,23,24). These disturbed processes can ultimately result in the displacement of cells with self-renewal capacity into the lamina propria and lead to the formation of signet ring cell carcinomas with the capacity for sustained cell division and thus to progression (19).

Identification of new genes underlying hereditary gastric cancer

In approximately two thirds of families fulfilling the strict HDGC criteria, no *CDH1* mutation is found and they remain genetically unexplained. Most of these families might carry mutations in other, still to be identified, GC susceptibility genes. As binding partner for E-cadherin, mutated β- and γ-catenin have been considered as candidates for diffuse GC predisposition (25). The β-catenin gene (*CTNNB1*) was recently assessed in a series of 40 families with positive history of GC from the Netherlands without finding any mutations (Vogelaar et al., unpublished data, 2012).

Also in families with intestinal type GC exhibiting an autosomal dominant inheritance pattern, genetic susceptibility genes may play a role. No gene has been associated with this type of GC yet. In carefully selected patients next generation sequencing based techniques that allow for exome or even genome wide detection of genetic aberrations, might be exploited to unravel genetic predisposition in an unbiased way.

Prophylactic total gastrectomy in *CDH1* mutation carriers

Prophylactic gastrectomy is currently the only option to eliminate risk of GC development in *CDH1*mutation carriers (30). The prognosis of patients with a prophylactic gastrectomy is very good. The estimated overall mortality for total gastrectomy is 2–4% with a nearly 100% risk of long-term morbidity. Associated problems following gastrectomy include abdominal pain after eating, dumping syndrome, lactose intolerance, fat malabsorption and steatorrhoea and postprandial fullness (8,13,14,28). The optimal timing of prophylactic gastrectomy in

individuals with *CDH1*mutations is not yet known. Preventive gastrectomy specimens of *CDH1* mutation carriers reveal multiple small signet ring cell lesions with low proliferation rates; few of these lesions progress to an aggressive carcinoma beyond the muscular mucosa (2). It is unknown why only some of these lesions develop into aggressive carcinomas. No correlation between patient age and number of small signet ring cell foci has been observed. Blair *et al.* advise *CDH1* mutation carriers with normal gastric biopsies to consider gastrectomy once the individuals are older than 20 years of age (1). Other authors recommend considering preventive gastrectomy when the *CDH1* mutation carrier is 5 years younger than the youngest family member with DGC, which generally means that preventive gastrectomy is postponed to an age later than 18 years (6).

In case of a preventive gastrectomy, total gastrectomy with Roux-en-Y reconstruction is recommended. There is no need for a radical lymph node dissection in the prophylactic setting since mucosal adenocarcinomas without submucosal invasion have a low risk of lymph node metastases (34).

Surveillance endoscopy

The 'Cambridge surveillance protocol' is advised for *CDH1* mutation carriers who do not want to undergo a prophylactic gastrectomy, to individuals at 50% risk of being carrier who are not willing to be tested for the mutation as well as for members from HDGC families without a known *CDH1* mutation (22). This protocol comprises *H.Pylori*-testing, annual gastroscopy with 'high definition' endoscope, careful inspection of mucosa during 30 minutes, insufflation and desufflation of the stomach, biopsies of mucosal abnormalities and 30 random biopsies from different gastric regions (antrum, angulus, corpus, fundus, cardia) (13). The endoscopy should be performed using a white light high definition endoscope in a dedicated session with at least 30 minutes allocated to allow for a careful inspection of the mucosa on inflation and deflation, and to allow time for multiple biopsies to be taken (13).

Use of mucolytics such as acetylcysteine may be helpful to obtain good views. Endoscopy permits direct inspection and biopsy of suspicious areas, but diffuse GC is difficult to detect at an early and treatable stage since the lesions tend to spread into the lamina propria without visible exophytic masses. The major problems include difficulties to identify (sub)mucosal lesions and biases in sampling in macroscopically normal-appearing gastric mucosa (15). Such specimens therefore need to be evaluated by pathologists with expertise with this type of lesions. Several studies have shown that even though *CDH1* mutation carriers had negative biopsies prior to prophylactic gastrectomy, foci were detected in their gastrectomy specimens(2,7,19,30). Other techniques, such as chromoendoscopic techniques, trimodal imaging, confocal endomicroscopy and molecular imaging techniques are currently not recommended, but need to be further explored in a research setting (13).

CONCLUSIONS

The overall incidence of GC is declining, which is most likely due to the reduction in environmental risk factors. A positive family history is a strong and consistently reported risk factor for gastric cancer. Germline mutations in the *CDH1* gene have been identified as an important cause of HDGC, but still in more than two thirds of strictly selected HDGC families the genetic cause remains unknown. Elucidation of novel gastric cancer susceptibility genes will be an important step towards additional options for gastric cancer prevention. Therefore, identifying new genetic gastric cancer predisposing factors is one of the important targets in the near future.

Conflict of interest: None

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CASE REPORT

Expression of Insulin-like Growth Factor 1 (Igf1) and its Receptor (Igfr1) in Two Extremely Pre-Term Placentas

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Summary
Today extremely premature children readily survive; good quality of life can be reached only with research and technology based approaches, therefore understanding of molecular processes taking place in pre-term placentas can be of practical value both for neonatology and pediatric surgery, as complications of prematurity quite often require surgical interventions. The present case report describes the expression of one of the most significant growth factors IGF1 and its receptor IGFR1 in placentas after extremely pre-term deliveries of 22 and 23 gestational weeks and their correlations with clinical findings of corresponding extremely premature children. Significantly more beneficial clinical course is represented by a child with clinically less advantageous situation: smaller gestational age (22w versus 23 w) and birthweight (540g v. 650g), born in the first vaginal delivery (v. repeated delivery) and recently ruptured membranes (v. PPROM > 72 hours). Placental cells of this child contained abundance of IGF1 and IGFR1 positive structures (v. few structures in the other one), possibly revealing better protective features of placenta, improving survival capabilities of the neonate in cases of extreme prematurity.

AIM OF THE DEMONSTRATION

The survival and good quality of life of extremely prematurily born children is one of the most challenging tasks of neonatology; its fulfillment requires research based approaches. Takizawa et al. (1) in 2007 suggested immunohistochemistry (IHC) to be useful for the clinical praxis. We evaluated the expression of 11 markers in the post-delivery placentas of different gestational ages, including the most potent growth factor, described by Peters et al. in 2012 (2) as a diagnostic marker of growth hormone deficiency, IGF1 and its receptor IGFR1. IGF1 and IGFR1 have been found in the placentas of various gestational ages; Kumar et al. (3) in 2012 found down-regulation of IGF1 with an advancing gestational age, alerting its possible role in extreme prematurity. We also found negative correlations of the expressions of IGF1 and IGFR1 with the gestation; just one of the extremely pre-term post-delivery placentas showed unusually weak immunoreactivity, correlating with the clinical findings. Clinically interesting was comparison with another case with a strong immunoreactivity of IGF1 and IGFR1. The other researched molecular markers in those two placentas were not so different.

CASE REPORT

In 2011, an IHC examination of 53 placentas of various gestational ages, acquired in the Riga Maternity hospital, was performed at the Latvian Institute of Anatomy and Anthropology. The study was approved by the Ethics comittee of the Riga Stradins university. Samples from identical central and peripheral places of placentas were taken immediately after delivery and placed into preservative; processed by antibodies IGF1 (mouse monoclonal, 1: 50, R&D) and IGFR1 (goat polyclonal, 1: 100, R&D); assessed visually by the same researcher at the same day; amount of cells, containing markers, were evaluated in the range from 0 (none) to ++++ (abundace) (4). IGF1 and IGFR1 mainly were contained by the cells of cytotrophoblast and extravillous trophoblast.

Two placentas: 22w and 23w revealed significantly different expressions of IGF1 and IGFR1 (Figure 1).

Case A: mother 20 years of age, normal 22w pregnancy, first vaginal delivery, a girl of 540g. No antenatal corticosteroids. The girl presented 2nd grade intra-ventricular hemorrhage (IVH) and moderate respiratory distress syndrome (RDS). Unusually benign clinical course for the gestational age.

Case B: mother 36 years of age, normal 23w pregnancy, PPROM > 72 hours, third vaginal delivery of a boy of 650g. No antenatal corticosteroids. The boy presented 3rd grade IVH and severe RDS, died on the third day of life due to extreme prematurity.

DISCUSSION

Clinically cases A and B were somehow similar: mothers had similar social statuses, body composition (body mass index 24 and 21), no high risks of infections or pre-term deliveries. Both pre-term delieveries were vaginal in the gestation of 22 and 23 weeks, without antenatal prophylaxis of dexamethasone, birthweight of children 540 and 650 grams.

Other studies have shown correlations of IGF1/IGFR1 in the placental tissues with the weight of the neonate: Iniguez et al. (5) found higher expression of IGF1 and IGFR1 in the placentas of small for gestational age (SGA) infants. In our case both of the children were appropriate for their gestational age; placenta A had much stronger expression of IGF1 and IGFR1 (abundant ++++) and a child with a lower Ponderal index (PI) of 2.0; placenta B had much weaker (occasional 0/+) expression of IGF1 and IGFR1 and a child with a higher PI (2.96). In this gestational age leanness (lower PI) could be underataken as disadvantage, in our case it was the beneficial case.

Preterm premature rupture of membranes (PPROM) have been described in 2012 by Blumenfeld et al. (6) as decreasing mortality among children of 24 to 26 weeks of gestation; in our case child A without PPROM showed better survival. Loukovaara et al. (7) in 2002 found no differences of the expression of IGF1 in the cases of PROM; probably PPROM had no impact on the expression of IGF1 in our case. Our findings could be more correlative with studies described by Isgaard et al. (8) in 2007 on the capability of IGF1 to protect and regenerate human brain.

We concluded that the expression of IGF1 and IGFR1 in the placental cells probably indicate the survival capabilities of pre-mature newborns; further studies could suggest application of IGF1 in the management of extremely low birth weight infants.

Conflict of interest: None

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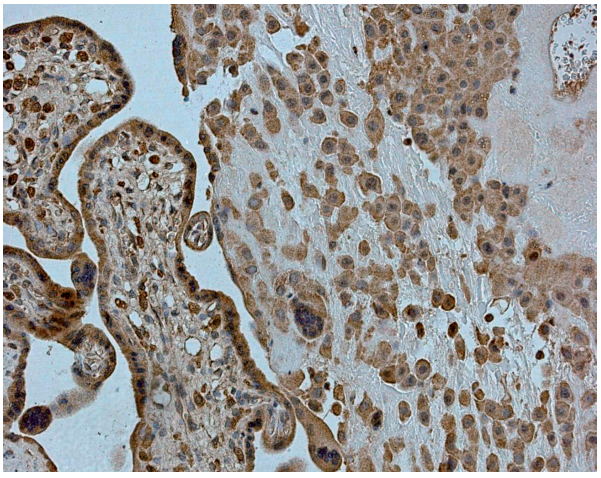

Fig. 1. Placenta A. IGF1 IHC, X 250

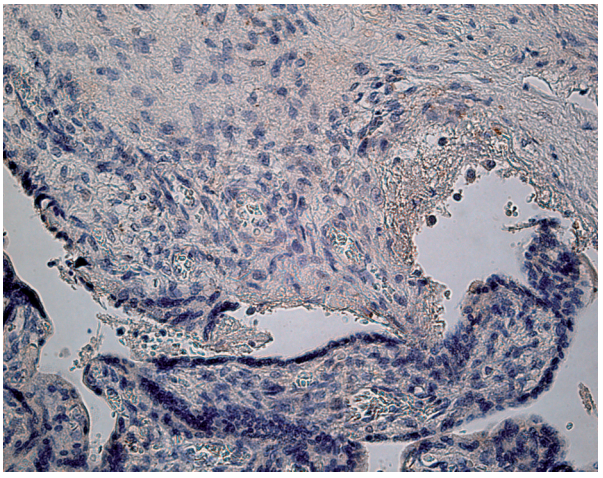

Fig.2. Placenta B. IGF1 IHC, X 250

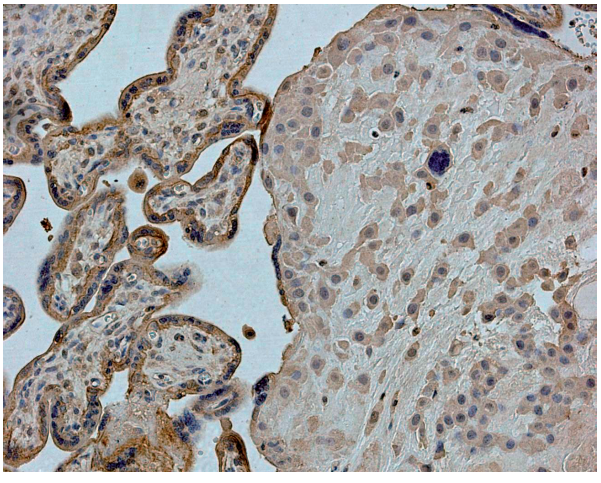

Fig. 3. Placenta A. IGFR1 IHC, X 250

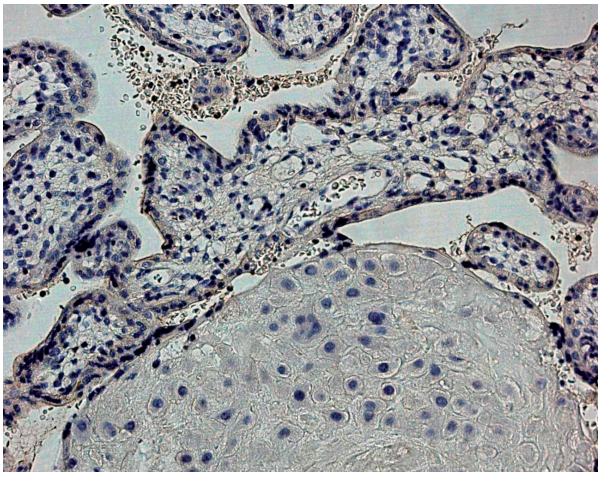

Fig. 4. Placenta B. IGFR1 IHC, X 250

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ACTA CHIRURGICA LATVIENSIS • 2013 (13)

CASE REPORT

Application of Cervicocapital Endoprosthesis in Treatment of Pathological Fracture of the Humerus

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Summary
Osteosarcoma is one of the most common malignant tumours of osseous tissue, where localisation of the tumour in the proximal third part of the humerus is the third most frequent (6). Prosthesing of the teenager's right humerus after pathological fracture was done with cervicocapital or Moore endoprosthesis. This endoprothesis developed for the treatment of femoral neck fracture and using for hip hemiarthroplasty. After 10 years the patient has suffered fracture of periprosthesis, osteosynthesis has been performed. Within 2 months after osteosynthesis the patient has regained the previous functional level without complications.
Key words: osteosarcoma; pathological fracture; cervicocapital or Moore endoprosthesis.

AIM OF THE DEMONSTRATION

Application of cervicocapital endoprosthesis in an atypical site, longterm results and their analysis.

CASE REPORT

A male, 25, was admitted to of the Traumatology and Orthopedics Hospital on 15.12.2012 with low energy trauma of the right upper arm after falling. On examination fracture of the distal third of periprosthesis of the upper arm with fragment displacement was detected.

The patient has had osteosarcoma since 2002. Biopsy was done in the Children Clinical University Hospital, the patient received the chemotherapy course. In 2003 the patient suffered from pathological fracture of the proximal third of the right upper arm. On 07.05.2003 resection of osteosarcoma of the upper arm bone was performed, and due to lack of the corresponding implant, endoprosthesing with cervicocapital endoprosthesis was performed (Fig.1). Histologic finding on 13.05.2003: highly differentiated osteosarcoma of the upper arm bone.

After trauma on 15.12.2012 corresponding osteosynthesis construction was ordered. On 02.01.2013 after receiving of the construction, osteosynthesis was performed. Osteosynthesis with 2 interlocked plates (DHP 2.7/3.5, right dorsolateral 14 holes and LCP Metaplys PL 3.5 f/dist.med.humer 13 holes) medially and laterally, Tomofix 3.5 screws and clamp loops. The patient withstood the operation adequately to its severeness and the type of anaesthesia. Physiotherapy was started (Fig.2). Sensation and movements of the fingers were not disturbed. Examination of the patient 2 months after osteosynthesis reveals the patient's condition corresponds to the operation performed. The patient is able to perform the previous activities and feels well, neurologic disturbances in the right arm have not been detected. Abduction 5 degrees, adduction 0 degrees, flexion 5 degrees, extension 5 degrees, external rotation 25 degrees and internal rotation 15 degrees.

DISCUSSION

Osteosarcoma is an extremely agressive and malignant tumour of osseous tissues with bad prognosis. For its treatment mainly chemotherapy and surgery are used (3). Surgical treatment includes several variations. Surgical treatment is more complicated if the tumour is localised in the area of joints, since in this case not only arm salvage is important, but also maintenance of its functions (6).

Reconstruction with endoprothesis Modular Replacement System (MRS), which was stabilised with Dacron tapes at the clavicle and shoulder blade and muscular transposition.Using this methode in 23 patients after 10 years 15 patients had remission, the prosthesis still functions in 15 survivors. Elbow and forearm functions maintained, in 8 patients transitory neurapraxy has been observed (5). Performing sparing proximal humerus resection and endoprosthesing, for example, with MRS or another device, they can supplemented by Tikhoff – Limberg procedure or its modification (6). If resection involves all humerus, it is possible to use total humerus endoprosthesing together with both articulations (shoulder and elbow) (4). Autotransplantation is possible as well, using avascular autofibula, if can saving humerus head. Excellent results have been acquired after 10 years, patient's range motion was minimally disturbed (2). A similar method of treatment was chosen for 8 patients having humerus tumours, who also were treated by avascular fibula transplantation. After 70 months 7 patients are still alive, 5 of them having satisfactory functions of the shoulder joint (7). Studies of 53 cases within 50 years revealed that chemotherapy in isolation did not significantly affect survival. Limb salvage surgery did not have an adverse effect on survival rates (3).

If limb salvage surgery with MRS or other method is not possible, it may be replaced by cervicocapital endoprosthesis. Cervicocapital endoprosthesing has good long-term outcomes in treatment of osteosarcoma of proximal humerus.

Conflict of interest: None

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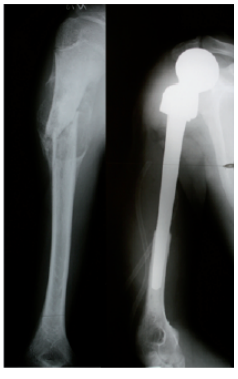

Fig. 1. Right humerus
Left side – right humerus pathological fracture
Right side – replacement with cervicocapital endoprothesis

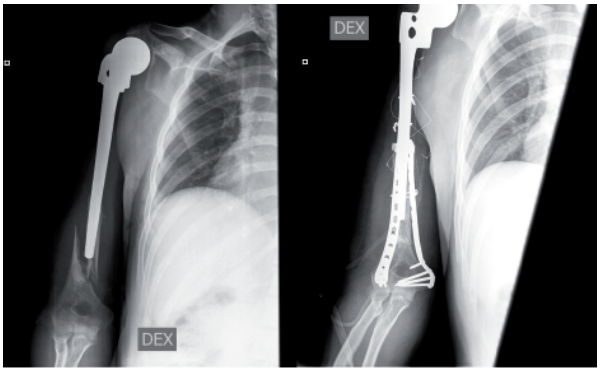

Fig. 2. Right humerus
Left side – periprothesic fracture
Right side – right humerus after osteosintesis

EIROPAS REĢIONĀLĀS ATTĪSTĪBAS FONDS

IEGULDĪJUMS TAVĀ NĀKOTNĒ

EIROPAS SAVIENĪBA