

METHYLATION ANALYSIS OF TUMOR SUPPRESSOR GENES IN OVARIAN CANCER USING MS-MLPA

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Introduction:

Ovarian cancer is the leading cause of death from gynecologic tumors due to its aggressive nature and the fact that the majority of patients are diagnosed in advanced stages of the disease. It has generally been believed that if ovarian cancer could be diagnosed at an early stage, this would result in a significant improvement in survival.

Aberrant methylation of normally unmethylated CpG islands, located in the 5' promoter region of genes, has been associated with transcriptional inactivation of several genes in human cancer, and can serve as an alternative to mutational inactivation [1].

A number of methods have been developed for detection of methylation alterations in tumors, such as MSP (Methylation-specific PCR), MS-MLPA (Methylation-specific Multiplex Ligation-dependent Probe Amplification), MS-HRM (Methylation-sensitive High resolution melting), DNA sequencing, microarrays and others. MS-MLPA represents a rather novel cost-effective and time-efficient method [2]. MS-MLPA is an ideal technique to use in FFPE (formalin-fixed, paraffin-embedded) samples. It permits simultaneous identification of epigenetic alterations in a predefined set of up to 25 genes.

Aims

The aim of this study was to evaluate the occurrence of hypermethylation of the promoter regions of individual genes using MS-MLPA in the samples of ovarian carcinoma comparing to non-malignant ovarian tissue.

Materials and methods:

FFPE samples from both ovarian adenocarcinomas and normal ovarian tissue were obtained from 109 women (69 patients with ovarian cancer, 40 patients with normal ovarium) treated at the Department of Obstetrics and Gynecology, University Hospital Hradec Kralove, Czech Republic. The samples of normal ovary were obtained from patients surgically treated for non-malignant diagnosis. DNA was extracted using a Qiagen DNA extraction kit.

The present study used the MS-MLPA - ME002 and ME004 probe sets (MRC-Holland, Amsterdam, The Netherlands). Probe sequences, gene loci and chromosome locations can be found at www.mlpa.com. The experimental procedure was carried out according to the manufacturer's instructions, with minor modifications. PCR fragments were separated and quantified by electrophoresis on an ABI 3130 capillary sequencer and analyzed using GeneMapper4.0 (both Applied Biosystems). Peak identification and values corresponding to peak size in base pairs (bp), and peak areas were used for further data processing. CpG universal methylated and unmethylated DNA (Chemicon International, Temecula, CA) were used in every run as controls.

Results:

In the present study we used the ME002 probe set to analyze 69 samples of ovarian cancer and 40 control samples. Using ME004 probe set we analyzed 44 samples of ovarian cancer and 30 control samples. Using a 15% cut-off (ME002 kit) for methylation we observed statistically-significant higher methylation in genes MGMT ($p=0.05$), PAX5 ($p=0.002$), CDH13 ($p<0.001$), WT1 ($p=0.045$), THBS1 ($p=0.048$), and GATA5 ($p=0.05$) in ovarian cancer patients than in the control group. Conversely, in gene ESR1 we observed a statistically-significant ($p<0.001$) higher methylation in the control group than in the ovarian cancer group. Using a 20% cut-off (ME004 kit) for methylation we observed statistically-significant higher methylation in genes NTRK1 ($p=0.008$), GATA4 ($p<0.001$) and WIF1 ($p=0.005$) in ovarian cancer patients than in the control group.

Discussion

The biological features of ovarian cancer are determined by the underlying molecular alterations of the tumor cells, including the epigenetic inactivation of tumor suppressor genes as well as mutations and deletions. It is now clear that *de novo* promoter methylation is a common mechanism for inactivation of tumor suppressor genes. The promoter methylation status has been reported in several human neoplasms.

We observed significantly higher methylation in genes MGMTa, PAX5, CDH13, WT1, THBS1, GATA5, NTRK1, GATA4 and WIF1 in the cancer group than in the control group, conversely, in gene ESR1 we observed a statistically-significant higher methylation in the control group than in the ovarian cancer group, indicating that promoter methylation of these tumor suppressor genes may play an important role in ovarian carcinogenesis. These genes could be used in future screening for ovarian cancer, because methylated DNA has been detected in body fluids of ovarian cancer patients, for example in plasma, and the level correlated reasonably well with methylation levels in tumor tissue [3]. This finding could also have implications for future chemotherapy based on epigenetic changes, because platinum resistance is strongly associated with methylation-induced silencing of various drug response genes and pathways [4].

Conclusions / Summary

In conclusion, our study showed that there are significant differences in promoter methylation in MGMTa, PAX5, CDH13, WT1, THBS1, GATA5, ESR1, NTRK1, GATA4 and WIF1 genes between ovarian cancer and control samples, suggesting the importance of methylation changes of these genes in ovarian carcinogenesis.

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DETECTION OF THE GENE ENCODING SURFACE LIPOPROTEIN LIPL32 OF PATHOGENIC *LEPTOSPIRA* WITH USING OF REAL-TIME PCR METHOD AND THE ANALYSIS OF THE TEMPERATURE OF MELTING

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Introduction:

Leptospirosis is one of the world's most common zoonosis, with an incidence of approximately 0.1 – 100 per 100,000 in populations [1]. The infective agents are aerobic spiral bacteria of the *Leptospira* genus. Leptospire are divided into 20 genomospecies, pathogenic leptospire fall into 13 genetic types [2,3].

The sources of infection in humans are usually water and damp substrates contaminated with the urine of reservoir animals, particularly rodents.

The incubation period ranges between 1-4 weeks. The disease can proceed with slight influenza symptoms up to states with hepatorenal failure, cardiac and respiratory insufficiency, haemorrhagic diathesis or aseptic meningitis [4].

Laboratory diagnosis depends on the typical two-phase course of the disease. In the first week of clinical symptoms it is recommended to examine the material obtained from patients by molecular biological methods. In the second week of the disease, special serological methods are used – the Microscopic Agglutination Test (MAT).

In our study, we have focused on the detection of the gene that encodes the superficial LipL32 lipoprotein with using of real-time PCR and analysis of the temperature of melting. LipL32 occurs only in pathogenic leptospire and its structure is highly conservative [5].

Aims:

Aim of this study was to introduce real-time PCR method detecting the gene for LipL32 into clinical practice of laboratory diagnosis of pathogenic leptospire. Further we focused on the determination of positive and negative analytical specificity, determination of the limit of detection, examination of 230 laboratory strains of leptospire gained from Royal tropical Institute in Holland, and evaluation of results of examination of biological materials obtained from patients suspicious on leptospirosis.

Materials and methods:

Materials:

For determination of positive analytical specificity of real-time PCR method 11 laboratory strains of pathogenic leptospire were used (*L. icterohaemorrhagiae* Fryšava, *L. copenhageni* Lebe, *L. grippityphosa* P125, *L. grippityphosa* Ž6, *L. sejroe* M84, *L. istrica* J20, *L. bratislava* Jez Bratislava, *L. Pomona* Šimon, *L. canicola* Hond Utrecht IV, *L. polonica* Poland, *L. sorexjalna* Sorexjalna. Negative analytical specificity was verified in laboratory strains of *Escherichia coli*, *Streptococcus pneumoniae*, *Borrelia burgdorferi*, CMV, *Leptospira biflexa*.

For determination of the limit of detection real-time PCR method dilution series for pathogenic strain *L. icterohaemorrhagiae* Copenhageni was prepared. It contained following

dilutions 10^6 , 10^5 , 10^4 , 10^3 , 10^2 , 10, 5, 2, and 1 copies of genome /1 mL of liquid biological material.

Further 230 laboratory strains of leptospires (218 from pathogenic, 7 from non pathogenic and 5 from unknown genomospecies) were examined.

Total of 455 biological materials (253× plasma, 121× urine, 72× liquor, 7× BAL, 2× sputum) from 295 patients suspicious on leptospirosis with using of real-time PCR method detecting the gene encoding surface lipoprotein LipL32 were examined.

Methods:

For the detection of DNA pathogenic leptospires with using of the in-house real-time PCR (SybrGreen I) on the Lightcycler 1.5 amplification primers LipL32-270F (25 bp) and LipL32-692R (26 bp) from the area of the gene for LipL32 (amplification product of the size of 400 bp) were applied. As a control of inhibition of DNA polymerase, the amplification of the housekeeping gene for β actin during usage of primers IC838-2 IC (32 bp) and IC838-1 IC (32 bp) was done and the amplification product of the size of 838 bp was obtained.

The on-line record of fluorescence of the product at a properly selected amplification temperature profile was realized at the end of every cycle at the wave length $\lambda = 530$ nm, the identification of the presence of DNA pathogenic leptospires was performed by the using of the analysis of the temperature of melting.

Results:

Results of examination of real-time PCR method detecting the gene encoding gene for LipL32 in 11 laboratory strains which occurred on territory of the Czech Republic were positive (positive analytical specificity), in negative analytical specificity was the result of examination always negative. The limit of detection 1 – 5 copies of genome was determined.

Total of 218 laboratory strains of pathogenic leptospires were determined as LipL32 positive, 7 laboratory strains of non pathogenic as LipL32 negative, and from laboratory strains of leptospires of unknown genomospecies 4 strain were LipL32 positive.

Of 295 patients examined with the real-time PCR method detecting the gene LipL32, 9 (3,1 %) persons were evaluated as being leptospira positive; of these 15 (3,3 %) positive biological materials were gained (9x urine, 4x blood and 1x liquor).

Conclusions:

The real-time PCR method detecting gene encoding the superficial lipoprotein LipL32 is a suitable, quick and sufficiently reliable method for the diagnostics of laboratory strains of pathogenic leptospires and of acute form of leptospirosis.

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IMPACT OF WEIGHT REDUCTION ON PLASMA OMENTIN-1 LEVELS IN OBESE PATIENTS WITH DIABETES MELLITUS TYPE 1

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Introduction

Diabetes mellitus type 1 (DM1) is a chronic disease characterized by the absolute lack of insulin, resulting from the autoimmune destruction of pancreatic β cells (1). Obesity is typically related to diabetes mellitus type 2, although it has become a serious problem even for the group of type 1 diabetics (2). Redundant fat mass produces a wide range of cytokines - adipokines. Omentin-1 is a novel adipokine primarily expressed in the visceral adipose tissue(3). Plasma levels of omentin-1 are decreased in insulin resistant and proinflammatory states. Recently, a possible role in vasodilatation was suggested, as well as an antiinflammatory effect in the pathogenesis of atherosclerosis (4).

Aims

The aim of this study was to characterize omentin-1 plasma levels in obese patients with diabetes mellitus type 1 and to elucidate the relationship between the reduction of fat tissue, insulin resistance and omentin-1.

Methods

The study group of obese patients with diabetes mellitus type 1 ($n=14$, BMI $>30\text{kg/m}^2$, age 29-62 y, male/female~ 9/5) was recruited from the registr of diabetics of University Hospital in Hradec Králové. Plasma omentin-1 levels were estimated by ELISA (Enzyme-Linked Immunosorbent Assay, BioVendor, Heidelberg); **Phase I**. Measurements were repeated a **month after** (a week of fasting during hospital setting and three weeks on a diet containing 150g saccharides per day) and **year after** on a diet with 225g saccharides per day; **Phase II-III**. Total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triacylglycerols (TAG) were estimated enzymatically. The data were statistically analyzed by software Sigma Stat (One way ANOVA repeated measurement). The data are presented as average \pm standard deviation.

Results

During the weight reduction program BMI (body mass index) of the obese diabetics significantly ($P<0.001$) decreased ($32.7 \pm 1.83 \text{ kg/m}^2$ before vs. $31.3 \pm 1.9 \text{ kg/m}^2$ year after). Daily doses of insulin were lowered, long-term changes in lipid profile and cholesterol metabolism markers were detected. Omentin-1 plasma levels were stable during the Phase II ($5.1 \pm 2.2 \text{ ng/ml}$ before vs. $5.7 \pm 2.9 \text{ ng/ml}$ month after) but increased significantly in Phase III ($9.5 \pm 2.6 \text{ ng/ml}$ year after, $P<0.001$). Plasma omentin-1 levels significantly correlated with HDL ($r=0.44$, $P=0.005$), TAG ($r= - 0.41$, $P=0.011$).

Discussion

During the weight reduction program, BMI and therapeutical daily insulin dose of study group significantly decreased. Omentin-1 plasma levels were stable in Phase I-II, although the body weight of the study patients rapidly decreased during this period. In Phase III the significant increase in omentin-1 levels was detected, even if the body weight mildly increased in comparison with the Phase

II. The plasma omentin-1 levels dynamic does not follow BMI or FTM in short term changes. In our study, the long - term observation showed significantly higher plasma omentin-1 levels while lowered need of daily therapeutical doses of insulin, which could be indicative of increase in insulin sensitivity during the program. Plasma levels of HDL rised significantly, whereas plasma LDL decreased. The possitive correlation on omentin-1 with HDL and negative correlation with TAG plasma levels was found.

Conclusion

Diet and regime changes can influence plasma omentin-1 levels, however the plasma omentin dynamic does not seem to reflect acute weight reduction and is more likely related to the long-term tissue changes. Present results suggest the close relationship of omentin-1 plasma levels to insulin resistance and cholesterol metabolism.

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THE RESPONSE OF DENTAL PULP STEM CELLS AND DERMAL FIBROBLASTS TO MITOXANTRONE TREATMENT

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Introduction

Dental pulp stem cells (DPSCs) are population of mesenchymal stem cells (MSCs) with many unique properties such as ability to self-renew, capability to produce different cell types and high proliferative potential. DPSCs are relatively easily available source of MSCs which may be useful in clinical applications. In this study we characterized the response of DPSCs under genotoxic stress caused by chemotherapeutic agent mitoxantrone (MTX) and compared it with the response of normal human dermal fibroblasts (HDFs). We focused on the effects of MTX on viability and proliferation, cell cycle changes and reaction of proteins determining cell fate.

Methods

We isolated DPSCs from impacted third molars obtained from healthy donors undergoing tooth extraction for orthodontic reasons, as described in the study of Suchánek et al, 2007. Normal human dermal fibroblasts isolated from normal human juvenile foreskin were purchased from PromoCell (Heidelberg, Germany). Proliferation of affected cells was detected by Z2 Counter and viability by Vi-Cell XR using Trypan blue exclusion staining. Cell cycle changes were analyzed by flow cytometry. The activity of β -galactosidase was detected by Senescence β -galactosidase Staining Kit and γ H2AX foci by immunocytochemistry. Induction of apoptosis was determined by monitoring of caspases activity by Caspase-Glo Assays. Protein levels were evaluated by electrophoresis and Western blotting.

Results

MTX provoked an increase in p53 and its phosphorylation of serine 15 as well as the expression of p21^{WAF1/Cip1} in DPSCs and HDFs. Treatment with MTX resulted in stress-induced premature senescence, we observed the accumulation of affected cells in G2 phase, enhanced activity of senescence-associated β -galactosidase and persisting DSBs-associated γ H2AX foci in both cell lines. In HDFs, we detected an increased expression of cell-cycle inhibitor p16^{INK4a}, which is responsible for permanent cell cycle arrest. Interestingly, the expression of p16^{INK4a} was not observed in DPSCs. Higher concentrations of MTX (> 50 nmol/l) induced a significant increase in caspase 3/7 activity and caused decrease in the viability of DPSCs as well as in HDFs.

Discussion

In some types of cell lines, predominantly mesenchymal, DNA damage leads to stress-induced premature senescence (SIPS). In the study of Robles and Adami (1998), the exposure

of HDFs to DNA strand breaking agents, bleomycin and actinomycin D, results in increased levels of p16^{INK4a} and in SIPS 8 days after application of these agents. Also it was found, that human diploid fibroblasts exposed to other genotoxic agents, such as hydrogen peroxide (Chen et al., 1998, Fripiat et al., 2001), tert-butylhydroperoxide (t-BHP), ultraviolet B radiation (Debacq-Chainiaux et al., 2005) or ethanol (Dumont et al., 2000) undergo SIPS. We have found that also application of MTX, widely used chemotherapeutic agent, cause SIPS in our tested cell lines. There was one apparent difference in the response of HDFs and DPSCs to MTX treatment. In the case of MTX-affected HDFs, we observed the increased expression of p16^{INK4a}, but we did not detect the activation of p16^{INK4a} in DPSCs during whole experiment (1, 3, 6 and 9 days after MTX application). These results are in contradiction with the work of Muthná et al. (2010), who observed increased expression of p16^{INK4a} after irradiation of DPSCs. In their study, the expression of p16^{INK4a} occurred already 24 hours after irradiation and its level increased for 13 days. Similarly, irradiation of hematopoietic stem cells induces premature senescence by activation of p16^{INK4a} pathways (Wang et al., 2006). It is not known why MTX did not activate p16^{INK4a} pathways in DPSCs. The fact that protein p16^{INK4a} is not involved in the process of SIPS in DPSCs points out complexity of molecular networks. It is possible that MTX activates different pathways to induce a stable senescence-like state in DPSCs. Thus further careful study is needed to elucidate this finding.

Conclusion

In summary, exposure of DPSCs and HDFs to clinically relevant concentrations of MTX resulted in stress-induced premature senescence. The reaction of DPSCs was not comparable with HDFs in all aspects. In DPSCs, MTX did not provoke increase in expression of cell-cycle inhibitors such as p16^{INK4a}, p15^{INK4b}, which are considered to play the main role in the permanent cell cycle arrest. It seems that other pathways contribute to the process of SIPS in MTX-treated DPSCs. Higher concentrations of MTX induced caspase-mediated apoptosis in both cell lines.

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EGFR IN TRIPLE NEGATIVE BREAST CARCINOMA; ARE WE BARKING UP THE WRONG TREE?

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Introduction

Breast cancer is a heterogenous group of malignancies comprising phenotypically and genetically distinct entities. Up to 20% of breast carcinomas are 'triple negative'; that is, they lack estrogen and progesterone receptor expression and do not over-express HER2 therefore they are unlikely to respond to hormonal or HER2 targeted therapy (1). Triple negative breast carcinomas (TNBCs) are associated with poor prognosis and yet paradoxically, reports show that some of them respond very well to neoadjuvant chemotherapy (1, 2). There is currently no standard approach to management of these tumors or any widely accepted evidence-based and clinically relevant way to sub-classify them.

EGFR has been put forward as a possible target molecule for biological therapy of TNBC. Clinical trials are currently underway to determine the effects of EGFR targeted in patients with this type of malignant breast disease. So far treatment with monoclonal antibodies has not produced satisfactory results (3, 4).

The purpose of this study was to assess and compare immunohistochemical (IHC) expression of EGFR and in-situ hybridization detection of EGFR gene and chromosome 7 in cases of TNBC and to discover possible clinico-pathological correlations.

Methods and Materials

From the archives of the Fingerland Department of Pathology, we randomly selected 37 cases of newly diagnosed pre-treatment TNBC for which adequate material for further studies and sufficient clinical data were available. Triple negativity was defined as immunohistochemical IRS (immunoreactive score) = 0 for estrogen and progesterone receptors with HER score = 0, 1+ or 2+ non-amplified by fluorescent in-situ hybridization (FISH) (chromosome 17:HER2 gene ratio < 2). In each case one representative formalin-fixed paraffin-embedded tissue block was selected for further studies.

A clinical chart review was also performed to determine oncological history, significant co-morbidity, breast cancer management and clinical course during the follow-up period. Where available the pathological stage at time of diagnosis (pTNM) was also obtained. For the cases in which the patients received neoadjuvant chemotherapy, clinical stage (TMN) at the time of diagnosis was recorded instead.

Immunohistochemical (IHC) staining for detection of EGFR was performed using the EGFR pharmDx™ Kit and according to the manufacturer's guidelines. A semiquantitative method for scoring EGFR expression was used employing a combination of staining intensity and percentage of positive tumor cells. Intensity was graded on four levels – 0 (no cytoplasmic membrane staining), 1 (low), 2 (moderate) and 3 (high). Percentage of positive cells was scored as follows 1 (≤10%), 2 (11-50%), 3 (51-80%) and 4 (>80%). These 2 parameters were then combined to give a final EGFR score (1-12).

Dual in-situ hybridization (dual ISH) assay was performed using the INFORM EGFR DNA probe assay and Ventana Alk Phos Red ISH detection kit for chromosome 7 centromere. For each case, the numbers of copies of EGFR gene and chromosome 7 were counted and recorded in 40 different tumor cell nuclei. The average number of copies of the gene and chromosome for each case was recorded and the gene-chromosome copy ratio was calculated.

Results

All the patients were women aged 28-83 years at the time of diagnosis (average: 53 years, median: 53 years). The average duration of follow-up was 52 months (range: 2-87 months, median: 58 months). At the end of the follow-up period, 27/37 patients were disease free and 5/37 had developed distant metastases (all within 24 months of initial breast cancer diagnosis). All the patients with ≥ 4 EGFR gene copies per cell were disease free at the end of the follow up period.

Results of IHC and ISH staining are summarized in tables 2 and 3 with clinical correlations shown in tables 3 and 4. Gene copy number increased with increasing EGFR score and amplification was seen in only one case and was not associated with poor outcome.

Table 1 EGFR gene copy number and immunohistochemical EGFR score

		EGFR gene copy number/cell			
		2-2.99	3-3.99	≥ 4	average
EGFR score					
0	(7)	7	0	0	2.51
1-3	(8)	4	2	2	3.09
4-6	(13)	7	4	2	3.10
7-9	(6)	2	3	1	3.29
10-12	(3)	1	0	2	6.38

Table 2 EGFR score and tumor clinico-pathologic characteristics

	EGFR score				
	0 (n=7)	1-3 (n=8)	4-6 (n=13)	7-9 (n=6)	10-12 (n=3)
Stage					
I	2	4	5	1	1
II	3	2	4	4	0
III	2	1	2	0	1
IV	0	1	2	1	1
Outcome					
Disease free	5	6	10	4	2
Locoregional disease	0	0	2	0	0
Metastasis	2	2	1	1	0
Death	0	0	0	1	1

Table 3 EGFR gene copy number and outcome

	EGFR gene copy number/cell		
	2-2.99 (n=21)	3-3.99 (n=9)	≥ 4 (n=7)
Outcome			
Disease free	16	4	7
Locoregional disease	1	1	0

Metastasis	3	3	0
Death	1	1	0

Discussion

EGFR is a receptor for multiple ligands and its activation leads to a wide range of biological responses from apoptosis and migration to proliferation and dedifferentiation (5). EGFR expression has been described in 14-43% of all breast carcinomas (6, 7, 8) and in up to 60% of TNBCs (9). This finding has been correlated with poor prognosis, particularly in node-negative cases.

While the consequences of EGFR expression in breast cancer remain controversial, it remains an attractive candidate for targeted biological therapy, particularly for TNBC, provided a suitable method for patient selection can be devised.

In our sample of TNBCs neither IHC detected expression of EGFR nor increased gene copy number was associated with worse outcome; not even in the only amplified case (chromosome 7:EGFR gene ratio > 2). In fact, all of the patients with gene copy numbers ≥ 4 were disease free at the end of the follow up period. This finding was not statistically significant, possibly due to the small size of our sample. It is however highly suggestive. EGFR over-expression, at least in a certain sub-set of TNBCs, could be a marker of good prognosis. Inhibiting EGFR in such tumors could have deleterious effects.

Conclusion

Our results suggest that intensity of immunohistochemical EGFR staining using our scoring system corresponds with EGFR gene copy number in triple negative breast carcinoma. Outcome in the patients with high EGFR scores and/or EGFR copy number was not worse than in patients with low EGFR scores. The fact that EGFR expression does not seem to be associated with worse outcome calls to question the suitability of EGFR as a target molecule for therapy of TNBC.

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NT-proBNP LEVELS ON ADMISSION PREDICTS PULMONARY HYPERTENSION PERSISTENCE IN PATIENTS WITH ACUTE PULMONARY EMBOLISM

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Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is a rare, but due to unfavorable prognosis, feared complication of thromboembolic disease. We assessed the incidence and risk factors for pulmonary hypertension (PH) in a cohort of consecutive patients admitted with pulmonary embolism to the tertiary University Hospital. Data on the incidence of CTEPH after pulmonary embolism are inconsistent. Relevant studies of patients surviving symptomatic pulmonary embolism showed CTEPH incidence in the range of 0,1%–8% [1].

Aims

Our prospective study was designed to evaluate possibilities of CTEPH prediction in a population of consecutive patients with pulmonary embolism, admitted to tertiary department of cardiology and to determine CTEPH incidence in our study population.

Methods

In our cohort of 120 consecutive patients admitted to our department from July 2007 to March 2010 with proved pulmonary embolism (PE) we studied the course of biochemical and echocardiographic parameters with regard to risk factors predicting pulmonary hypertension at the end of hospitalization. In most cases the PE diagnosis was based on multidetector computer tomography angiogram (CTA) using Siemens Somatom Emotion 6. On admission blood samples were assessed to determine troponin-T (TnT) and N-terminal fragments of brain natriuretic peptide precursor (NT-proBNP) levels (electrochemoluminescent method on Elexis device (Roche company)), a D-dimer (immunoturbidimetric method on device Compact STA-R (Stago company)). Within first 24 hours echocardiography was carried out (on PHILIPS SONOS 5500 or GE Vivid7), focused to right ventricle (RV) diameter, signs of RV systolic dysfunction (systolic excursion of lateral part of tricuspid annulus (TAPSE), peak velocity of this movement (SaTri), RV free wall hypokinesis, RV dilatation and pulmonary artery systolic pressure (PAsP) estimation. Biomarkers were reassessed and echocardiography was performed before discharge.

Results

During hospitalization there was a statistically significant ($p = 0.0014$) decrease of RV diameter, estimated pulmonary artery systolic pressure, improvement of RV function parameters and significant decrease of NT-proBNP levels. Pulmonary hypertension persistence at the time of discharge correlated with some variables on admission. A strong correlation was found between discharge PAsP and initial PAsP ($r = 0,701$). Also a positive correlation with initial NT-proBNP ($r = 0,443$) and age ($r = 0,46$) was found. Considering all variables obtained at the time before discharge, there was a correlation between elevated PAsP

and persisting elevation of NT-proBNP ($r = 0,42$) and RV dilatation ($r = 0,42$). Despite the relationship between discharge PH and elevated NT-proBNP as a marker of RV dysfunction, there was surprisingly no correlation with either echocardiographic signs of RV dysfunction, or reduction rate of RV diameter or PAsP during hospitalization.

Discussion/Conclusion

In our own cohort of patients with PE echocardiographic, signs of pulmonary hypertension at discharge time were present in more than one-half of patients (50,8 %). Respecting recent guidelines [2] those patients should be dispensarised with the focus to possible CTEPH development. According to our results patients requiring further follow up due to increased risk of PH persistence, could be indentified even at the time of admission, pursuant high initial NT-proBNP levels and echocardiographic signs of pulmonary hypertension, especially in the elderly patients.

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