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ASSESSMENT OF THE CARDIOTOXICITY INDUCED BY CHEMOTHERAPY. CORRELATIONS WITH THE CARDIOVASCULAR RISK FACTORS

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ABSTRACT

Objective/Purpose: Knowing the risk factors for chemotherapy-induced cardiovascular complication can help to focus preventive efforts to reduce cardiotoxicity. We assessed the association of cardiovascular risk factors with the appearance of cardiotoxicity in breast cancer patients.

Design and Method: A prospective study was carried out on 72 women with HER2-positive breast cancer. Inclusion criteria were: age > 18 year, normal sinus rhythm, left ventricular ejection fraction (LVEF) > 50%, HER2 overexpression and trastuzumab therapy. In all patients cardiovascular risk factors were quantified. For the SCORE risk assessment, were used the SCORE chart for the European zone with high risk of cardiovascular mortality. Metabolic syndrome was diagnosed according to the International Diabetes Federation criteria. According to the Seidman criteria recommended by the 2012 ESMO guidelines the patients were examined at baseline and then at 3, 6 and 12 months and the cardiotoxicity was diagnosed by decrease of LVEF.

Results: The mean age at baseline was 55.4 ± 11.80 years. Hypertension was present in 43.1% (n=31) patients and increased with 4.9% at 12 months ($\chi 2$ test, $p < 0.001$). The analysis of cardiovascular risk factors shows that metabolic syndrome was present in 33.3% of patients (n = 24), 12.5% of patients were diabetic, 37.5% were obese. The Spearman test revealed significant correlations between the decrease of LVEF with 5-10% and >10% and the total number of chemotherapy cycles. There was a significant correlation between SCORE risk and decrease of LVEF < 55% at 12 months (test $\chi 2$, p=0,001). Severe cardiotoxicity occurred in 3 patients with very high SCORE risk.

Conclusion: Assessment of cardiovascular risk factors for trastuzumab induced cardiotoxicity and appropriate patient monitoring during trastuzumab treatment allows for safe and effective use of this important adjuvant therapy.

Keywords: cardiotoxicity, chemotherapy, trastuzumab, risk factors, breast cancer

INTRODUCTION

Cardiovascular disease (CVD) and cancer are the most common diseases in our times, and continue to rise. Treatment for cancer has become more efficient, but instead, cardiac damage in these patients has become more frequent. CVD can affect the quality of patients life and the course of cancer treatment (1).

The frequency of breast cancer increases with age and has a higher incidence especially in premenopausal (between 45-49 years) and postmenopausal (> 60 years) according to European Society of Medical Oncology (ESMO) (2). Recently concerns have focused on identifying patients at risk of cardiac events due to chemiotherapy. So far, only a few risk factors have been identified among patients enrolled in clinical trials because most of them were in optimal health (3-5).

Cardiotoxicity may be the dose limiting factor in cancer treatment and appears during therapy with several cytotoxic agents. In addition, cardiotoxicity may also be responsible for the long-term side effects and may cause severe morbidity of surviving cancer patients (6).

Cardiotoxicity from chemotherapy is known to have a high prevalence (7) and more than 2 million breast cancer survivors in the United States are at risk (1).

More recently, the National Cancer Institute in the United States defined cardiotoxicity as the 'toxicity that affects the heart'. This includes the direct effect of therapy on the heart and also indirectly through changes in haemodynamic flow alterations or due to thrombotic events (8).

However, now that progress in terms of early diagnosis, therapy, and survival has been made, specific drugs and combinations of two or three different agents have emerged, and intervention is more and more more frequently used in the early stage; subsequent cardiotoxicity is a central problem (2), becoming a rising concern, for both cardiologists and oncologists (9).

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Cardiotoxicity remains the limiting factor for many type of oncology therapy and represents the focus of increased clinical research (10).

The incidence of cardiotoxicity dependens on various risk factors (eg, patient age,sex, the total dose of anti-neoplastic drugs, time of follow-up, family/personal history of cardiovascular disease, and mediastinal irradiation), as well as the criteria used to define the cardiotoxicity, ranging in studies from 5% to 65% of patients (11,12).

The cardiac dysfunction is defined as the onset of cardiac symptoms or an asymptomatic decrease in left ventricular ejection fraction (LVEF) of 10% or more (13).

Several approaches can be made in order to identify cardiac dysfunction such as clinical examination, echocardiography and measurement of cardiac biomarkers, as troponins and natriuretic peptides, (as an indicators of myocardial injury) (5).

The cardiotoxicity of trastuzumab did not appear immediately and was only evident in Phase III clinical trials (14). The first clinical trials have shown little or no indication of increased risk of cardiotoxicity in patients treated with trastuzumab (15,16). For example, in a Phase II study in women with refractory metastatic breast carcinoma, has been reported that no grade 3 or 4 of cardiotoxicity appears following the National Cancer Institute criteria common in patients treated with trastuzumab plus cisplatin for 8 weeks (16). One of the nineteen patients treated (5%) showed cardiomyopathy during the same phase of the study. However, the patient, in particular, have a number of risk factors for arrhythmia, including prior cumulative dose of anthracyclines (ANT) to 420mg/m^2, mediastinal irradiation, high blood pressure (hypertension) and diabetes (16).

The delay in identifying the cardiotoxic effects due to the fact that heart problems were not initially anticipated, cardiac monitoring was not performed in most studies, and even if it was performed, the results were inconsistent from one study to another. As such, it is very difficult to compare results across studies to identify risk factors that predispose to the occurrence CIT. Furthermore, it is difficult to compare cardiotoxicity induced by trastuzumab with cardiotoxicity related to other chemotherapeutic agents as the nature of cardiac dysfunction associated with all these agents has not been yet characterized comprehensively (15).

The potential for cardiotoxicity should be recognised before before initiating chemotherapy. The probability of cardiotoxicity should be recognized before initiating chemotherapy. Furthermore, the initial assessment of these patients should include: cumulative dose (especially for anthracyclines, mitomycin); the total dose administered during the day or cycle (cyclophosphamide, ifosfamide, 5-fluorouracil); the rate of administration (anthracyclines, 5-fluorouracil); administration schedule (anthracyclines); mediastinal irradiation; age; female gender; coadministration with other cardiotoxic agents; previous anthracycline chemotherapy; history or pre-existing cardiovascular disorders; electrolyte imbalance (hypokalemia and hypomagnesemia) (17).

As a result, we conduct this study to investigate the incidence and risk of cardiotoxicity in patients treated with trastuzumab. The objectives of the study was identification and quantification of cardiovascular risk factors in a group of patients with HER-2 positive breast cancer, the evaluation at 3, 6 and 12 months after the inclusion in the study, of the systolic and diastolic function parameters of the left ventricle using 2D transthoracic echocardiography (2D-TTE) in order to assess an early diagnosis of cardiotoxicity and the correlation between the risk factors taken separately (hypertension, metabolic syndrome) and SCORE risk, as a measure of cardiovascular risk accumulation and the progression of the ejection fraction as a measure of systolic function, framed by Seidman criteria, recommended by ESMO guidelines.

PATIENTS AND METHODS

Study Population

All consecutive women with breast cancer undergoing trastuzumab therapy, from January, 2010, to November, 2014, were considered. The study was conducted over a period of 12 months for each patient. Indication for trastuzumab therapy were early-stage and advanced or metastatic breast cancer, with HER2 overexpression. The patients were in records of Oncology Clinic, Surgical Oncology Clinic and Department of Radiotherapy of Municipal Hospital, Timisoara. The patients had different numbers of chemotherapy cycles with trastuzumab during the study.

The inclusion criteria were: age > 18 year, normal sinus rhythm, left ventricular ejection fraction (LVEF) > 50%, HER2 overexpression and trastuzumab therapy. The local ethics committee approved the protocol, and written informed consent was obtained from all patients in accordance with the Helsinki Declaration.

Study Protocol

The breast cancer was diagnosed and ordered according to the receptors status (estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)) and the TNM classification for staging breast cancer.

In all patients cardiovascular risk factors were quantified. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, ***taking into account the average obtained from three determinations or already present antihypertensive treatment according to the European Guidelines on Cardiovascular Disease Prevention in Clinical Practice 2012 (18)***. ***For smoking condition*** were ***noted***

the number of cigarettes per day or non-***smoker in the last 4 weeks. Type 2 diabetes was defined according to the*** **fasting plasma glucose ≥** ***126 mg/dL on two different determinations or*** treatment with oral antidiabetic agents ***based on World Health Organization (WHO) cr***iteria and those of the American Society of Diabetology (19). Hypercholesterolemia, another cardiovascular risk factor, is defined by the values of total cholesterol over 200 mg/dl according to National Cholesterol Education Program Adult The Treatment Panel III (NCEP ATP III). Recommended target level in primary prevention is <190 mg / dl and the values that exceeded this limit were considered to be a major risk factor. The use of normolipidemic medication was retained as a criterion for diagnosis (20). Hypertriglyceridemia was defined according to ACC/AHA guidelines. HDL cholesterol was also detected, which also was used to calculate LDL cholesterol by Friedewald formula.

For the SCORE risk assessment, were used the SCORE chart for the European zone with high risk of cardiovascular mortality. Based on the results, breast cancer patients were included in the four categories: SCORE risk<5%, 5-10%, 10-15% or > 15% (21).

Evaluation of metabolic syndrome was defined according to National Cholesterol Education Program criteria Adult Treatment Panel III (NCEP ATP III, 2001) and the ones developed by International Diabetes Federation (IDF, 2005) (22).

The patients were *cardiologically examined* at baseline and then at 3, 6 and 12 months, evaluations that included clinical examination, ECG and echocardiography, as ESMO guidelines recommend (5). A standard 12-lead ECG was performed at each evaluation. The QT time was corrected for heart rate (QTc) with Bazett's formula (QTc = QT/√RR).

2D echocardiography was performed on ultrasound system (Vivid 4 General Electric, C5-multidisciplinary ultrasound, Vivid 9, General Electric Milwaukee) and the measurements were made in 2D M - mode, Colour Doppler, and to the special cases, Tissue Doppler and 3D measurements were applied.

Left ventricular diastolic function was evaluated by measuring Doppler transmitral flow: the maximal velocity of the E and A waves (rapid filling and atrial filling), the ratio of early peak flow velocity to atrial peak flow velocity (E/A ratio; normal value >1), left ventricular end-diastolic diameter (normal value, 47 ± 4 mm), left ventricular ejection fraction (LVEF) and the isovolumic relaxation time (IVRT; normal value <100 ms).

Cardiotoxicity was defined according to the Seidman *et al.* criteria issued by 2012 ESMO guidlines (5) and is characterized by '1) decrease in cardiac LVEF that was either global or more severe in the septum; (2) symptoms of congestive heart failure (CHF); (3) associated signs of CHF, including but not limited to S3 gallop, tachycardia, or both; and (4) decline in LVEF of at least 5% to less than 55% with accompanying signs or symptoms of CHF, or a decline in LVEF of at least 10% to below 55% without accompanying signs or symptoms.

Statistical analysis

Statistical processing was performed using Excel software packages, SPSSv.17 and Epi Info 7. In all cases we used the significance threshold of 0.05 (5%), corresponding to a 95% confidence level.

RESULTS

In total, 72 women (mean age, 55.4±11.80 years) were enrolled. Characteristics of the study group at baseline showed that the age of the breast cancer patients included was between 27 and 78 years. The measured values of the parameters of the lipid profile revealed a slight hypercholesterolemia, total cholesterol with mean values of 208.7 ± 45.60 mg / dl, the extreme values of 120 mg/dl and 350 mg/dl and hypertriglyceridemia, with means of 161.8 ± 51.82 mg/dl, extremes included between 63 mg/dl and 382 mg/dl. The mean HDL-C was 43.3 ± 13.47 mg/dl, the extremes being at 26 mg/dl and 85 mg/dl. The mean LDL-C was calculated according to the Friedewald formula and was 132.1 ± 44.70 mg/dl, with ranges between 27 mg/dl and 294 mg/dl. The other metabolic parameters were within normal limits with an average of 0.9 ± 0.35 mg/dl for creatinine and 4.8 ± 3.65 mg/dl for uric acid (Table I).

Table I. Characteristics of the study group at baseline (n=72)

CHARACTERISTIC	MEAN ± DEV.STD.	MINIMUM VALUE	MAXIMUM VALUE
AGE, YEARS	55.4±11.80	27	78
TOTAL CHOLESTEROL (mg/dl)	208.7±45.60	120	350
TRIGLYCERIDES (mg/dl)	161.8±51.82	63	382
HDL-C (mg/dl)	43.3±13.47	26	85
LDL-C (mg/dl)	132.1±44.70	27	294
CREATININE (mg/dl)	0.9±0.35	0.5	2.5
URIC ACID (mg/dl)	4.8±3.65	2.7	8.5
GLYCEMIA (mg/dl)	87±18.93	72	197
SBP (mmHG)	129.2±17.14	90	180
DBP (mmHG)	75.8±14.30	50	105
BMI (Kg/m^2)	32.8±5.23	28.1	41.93
WAIST CIRCUMFERENCE (cm)	89±15.74	75	127

Blood pressure (BP) values were between 129.2 ± 17.14 mmHg for SBP and 75.8 ± 14.30 mmHg for DBP, with extreme values of 180 mmHg and 105 mmHg.

Most patients were overweight or obese, and this is reflected in the mean values of the BMI between 32.8 ± 5.23 kg/m^2, extreme 28.1 kg/m^2 and 41.93 kg/m^2 and in the mean values of the waist circumference of 89 ± 15.74 cm, extreme 75 cm and 127 cm, 25% of patients

were with stage I obesity (n = 18), 8.3% with stage II (n = 6), and 4.2% with stage III (n = 3) (Table II).

Table II. The percentage distribution of degrees of obesity at baseline (n = 72)

OBESITY	FREQUENCY	%
normal weight	45	62.5
class I obesity	18	25.0
class II obesity	6	8.3
class III obesity	3	4.2
Total	72	100.0

Most of the patients had stage II breast cancer (36.9%) and III (37.6%), and in terms of TNM, most of them were classified as forms of advanced neoplasia, 19.4% T3 and 25%T4, with positive lymph nodes in a proportion of 37.5% N1 and 26.4% N2. Distant metastasis rate was positive in 15.3%. 40.3% of patients (n = 29) had right breast cancer and 59.7% of patients (n = 43) left breast cancer.

Determination of hormone dependent on tumor blocks or biopsy revealed fragments ER sensitivity over 15% to 75% of patients (n = 54) and PR over 20% in 63.9% of patients (n = 46). Ki-67 proliferative index was over 20 in 81.9% of the patients (n = 59). HER2 overexpression as determined by immunohistochemistry, was positive in all patients, representing an inclusion criteria. 50% were HER2+2 patients and 50% of patients were HER2+3 (n = 36 per share).

The analysis of the types of chemotherapies applied to the study group, found that cyclophosphamide was used in a proportion of 80.6% (n = 58), anthracyclines in 98.6% (n = 71), taxanes in 75% (n=54) and anti-metabolites at a rate of 5.6% (n = 4). All patients received trastuzumab, this being a criterion for inclusion in the study.

The analysis of cardiovascular risk factors in the studied group

The analysis of cardiovascular risk factors in the studied group shows a prevalence of metabolic syndrome in the majority of patients, with a ratio of 33.3% (n=24) and also high BP, rating 43.1%. (n=31). Obesity was present in 37.5% (n=27), and smoking in 26.4% (n=9). Diabetes mellitus was present at 12.5% (n=9)(Table III).

Table III. The percentage representation of risk factors in the study group at baseline (n = 72)

RISK FACTORS	No.	%
Smoking	19	26.4
Diabetes Type 2	9	12.5
Hypertension	31	43.1
Obesity	27	37.5
Metabolic Syndrome	24	33.3

The percentage distribution of the SCORE risk was <5% (considered to be low) in a proportion of 34.7% (n=25), 5-10% (considered to be increased) in 16.7% (n=12), 10-15% in 5.6% (n=4), and very high> 15% in 43.1% (n=31)(Table IV).

Table IV. The percentage representation of the SCORE risk in the study group (n = 72)

SCORE RISK	FREQUENCY	%
< 5%	25	34.7
5-10%	12	16.7
10-15%	4	5.6
>15%	31	43.1
Total	72	100.0

The analysis of ejection fraction in the studied group

Analysing the evolution of the echocardiographic parameters in the studied group, was found that the mean ejection fraction (EF) was 58.1 ± 5.22% at baseline and 54.5 ± 6.37% at 12 months. At the time of chemotherapy initiation, the extreme values of the EF were set between 49% minimum and 75% maximum, and 25% minimum and 68% maximum 67% at 3 and 6 months, so that after 12 months, the minimum value of EF returned to the value of 40% and maximum 66% (Table V).

Table V. The evolution of LVEF in the 4 time points in the studied group (mean ± dev.std.)

VARIABLES	MEAN ± DEV.STD.	MINIMUM VALUE	MAXIMUM VALUE
EF at baseline (n = 72)	58.1±5.22	49	75
EF at 3 months (n = 71)	55.0±7.19	25	68
EF at 6 months (n = 69)	54.4±7.46	25	67
EF at 12 months (n = 69)	54.5±6.37	40	66

Analysis of the EF values at 12 months are represented in Table VI. There were recorded 3 deaths (4.2% of patients). These occurred in 2 patients who presented to the evaluation of 3 and 6 months, 25% decreased EF that was associated with cardiovascular pathology. A third death occurred at 6 months, with a preserved EF of 52%. Consequently, echocardiography measurements at 3 months were performed on 71 patients, and at 6 and 12 months were performed on 69 patients. A proportion of 5.6% of the patients (n=4) showed EF of between 40-44%, 19.4% (n=14) had an EF between 45-50%, and 70.8% of the patients (n=51) were presented with EF> 50%.

All three deaths occurred in patients who have EF decreased between 5-10% and over 10% from EF baseline, respectively, all the three patients were part of the subgroup of patients with EF <55% (Table VI). The heart failure in the first two cases and the malignant ventricular rhythm disturbances in the third case were the causes of death installed on the merits of cardiotoxicity.

Table VI. The percentage and numerical representation of deaths according to the categories of EF decrease

EF	DEATHS	
	NO	YES
EF>55%	33	0
	100.0%	0%
Decrease <5%	17	0
	100.0%	0%
Decrease between 5-10%	8	2
	83.3%	16.7%
Decrease >10%	11	1
	94.1%	5.9%

The analysis of the ejection fraction following Seidman criteria recommended in the 2012 ESMO guidelines

EF evolution analysis after the Seidman criteria recommended in the 2012 ESMO guidelines, showed that the subgroup of patients with EF>55% was 47.8% (n=33) of all 69 patients remaining in the study, due to the 3 deaths. In the subgroup of patients with EF <55%, 24.6% (n=17) showed a decreased LVEF <5% from baseline, 11.5% (n=8) showed EF 5-10% decrease from baseline, and 15.9% (n=11) showed decreased EF>10% from baseline (Table VII).

Table VII. The percentage and numerical distribution of EF evolution at 12 months from baseline (n = 69) according to criteria recommended by ESMO 2012 guidlines

EF	FREQUENCY	%
EF>55%	33	47.8
percentage decreased of the patients with EF at endpoint < 55%		
Decrease <5%	17	24.6
Decrease between 5-10%	8	11.5
Decrease > 10%	11	15.9

The correlation between the number of cytostatic preparations and percentage of decrease in EF at 12 months

The association between the number of cytostatic preparations and percentage of decrease in EF at 12 months were performed for each patient. The results are presented in Table VIII for n = 69 patients.

Table VIII. The percentage and numerical distribution of EF evolution at 12 months from baseline and number of cytostatic preparations (n=69)

EF	NUMBER OF THERAPIES / PATIENT	
	< 5	>5
EF>55%	**18**	**15**
	54.5%	45.5%
Decrease <5%	**5**	**11**
	31.3%	68.8%
Decrease between 5-10%	**4**	**6**
	40.0%	60.0%
Decrease > 10%	**0**	**10**
	0.0%	100.0%

The association between the percentage of decrease in EF and the number of therapies was significant ($\chi 2$ test, p = 0.018).

The analysis of shortening fraction

The rank values of diastolic dysfunction were significantly lower at 12 months (Wilcoxon test for paired values, p <0.001)(Table IX). Shortening fraction (SF) decreased from 35.6 ± 5.38% to 32.5± 7.9% (p <0.001). SF evolution compared at baseline and at 12 months is represented graphically in Figure 1.

Table IX. The analysis of the rank values of shortening fraction at baseline and at 12 months (n = 69). Wilcoxon test for paired values

PAIRED VALUES	MEAN	STANDARD DEVIATION	STANDARD ERROR
SF at baseline	35.6	5.38	0.63
SF 12 months	32.5	7.90	0.93

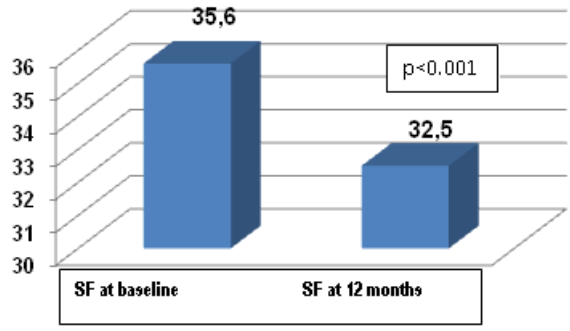

Fig.1. The graphical representation of SF values comparison at baseline and at 12 months. Wilcoxon test for paired values (n = 69)

The echocardiographic parameters of diastolic function in the studied group

In table X is represented the evolution of LV end-diastolic volume (EDV) in the study group (n = 72) during the four examinations: at baseline, at 3, 6 and 12

months (the endpoint of the study) as mean ± standard deviation. EDV average values were 71.6 ± 11.72 ml at baseline and 77.9 ± 19.96 ml at 12 months. EDV extreme values ranged between 42 ml initially (minimum) and 175 ml at 3 months (maximum). Due to the three deaths in the studied group. 3 months measurements were performed on 71 patients, and at 6 and 12 months on 69 patients.

Table X. The evolution of LV end-diastolic volume (EDV) in the study group (n = 72) during the 4 time points (mean ± dev.std.)

VARIABLES	MEAN ± DEV.STD.	MINIMUM VALUE	MAXIMUM VALUE
EDV at baseline (n = 72)	71.6±11.72	42	138
EDV at 3 months (n = 71)	76.9±22.70	55	175
EDV at 6 months (n = 69)	78.0±20.57	55	163
EDV at 12 months (n = 69)	77.9±19.96	51	157

EDV evolution from baseline to 12 months was observed by applying Friedmann non-parametric test of significance which showed significant differences between the values of EDV at the 4 time points (p = 0.002). Comparisons of the 2 by 2 points in time were performed with Wilcoxon Signed Ranks non-parametric test of significance and revealed the following results: at 3 months, EDV values significantly increased compared to the start (p = 0.001), at 6 months significantly raised from the ones at 3 months (p = 0.004) and to start (p <0.001) and at 12 months decreased slightly compared to the ones at 6 months (p = 0.421). EDV evolution compared at the 4 time points is represented graphically in Figure 2.

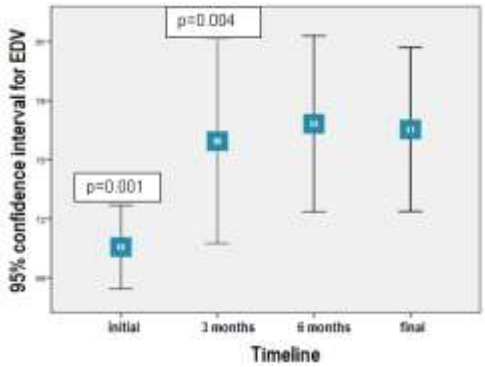

Fig.2. The graphical representation of mean values of EDV comparison at the 4 time points

For IVRT were used ordinal variables: 0 = 60-100 ms range values, 1 = values over the 100 ms and 2 = values of less than 60 ms. At 12 months the IVRT values were significantly increased (Wilcoxon test for paired values, p = 0.020) (Figure 3).

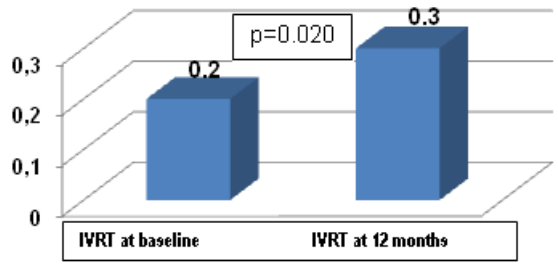

Fig.3. The graphical representation of IVRT values comparison at baseline and at 12 months. Wilcoxon test for paired values (n = 69)

Applying χ^2 test to these data, significant differences between the proportions of patients after initial and final IVRT group were observed (χ^2 test, p <0.001)(Figure 4).

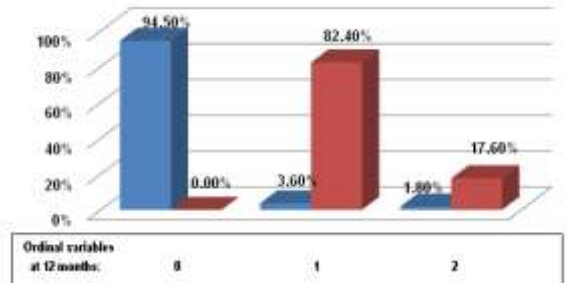

Fig.4. The graphical representation of the percentage differences between the distribution of patients after initial and final IVRT group (n = 69). χ2 Test

To analyze group differences for diastolic dysfunction were used ordinal variables: 0 = absence of diastolic dysfunction, 1 = Class I diastolic dysfunction, 2 = Class II diastolic dysfunction and 3 for Class III diastolic dysfunction.

Rank values of diastolic dysfunction were significantly increased at 12 months (Wilcoxon test for paired values)(Table XI). Comparison of diastolic dysfunction is represented in Figure 5.

Table XI. The analysis of the rank values of diastolic function parameters at baseline and at 12 months (n = 69). Wilcoxon test for paired values

PAIRED VALUES	MEAN	STANDARD DEVIATION	STANDARD ERROR
IVRT at baseline	0.2	0.43	0.05
IVRT at 12 months	0.3	0.58	0.07
Diastolic dysfunction at baseline	0.39	0.49	0.02
Diastolic dysfunction at 12 months	0.56	0.80	0.17

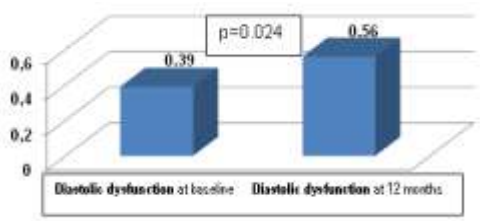

Fig.5. The graphical representation of the comparison of rank values between the parameters of diastolic function at baseline and at 12 months (n = 69). Wilcoxon test for paired values

The analysis of cardiovascular risk factors evolution

The blood pressure values at baseline were between 129.2 ± 17.14 mmHg for SBP, and 75.8 ± 14.30 mmHg for DBP, with extreme values of 180 mmHg for SBP and 105 mmHg for DBP. At 12 months, blood pressure values were between 130.0 ± 17.26 mmHg for SBP and 79.4 ± 12.29 mmHg for DBP, with extreme values of 170 mmHg for SBP and 105 mmHg for DBP (Table XII).

Table XII. The evolution of blood pressure in the study group at baseline and at 12 months. Mean and standard deviation

CHARACTERISTIC	MEAN ± DEV.STD.	MINIMUM VALUE	MAXIMUM VALUE
DBP at baseline (n = 72)	129.2±17.14	90	180
SBP at baseline (n = 72)	75.8±14.30	50	105
DBP at 12 months (n = 69)	130.0±17.26	90	170
SBP at 12 months (n = 69)	79.4±12.29	60	105

Wilcoxon test for paired values showed that the values of SBP were not significantly raised at 12 months compared to baseline (p = 0.537). DBP values were significantly increased at 12 months (p = 0.019)(Figure 6).

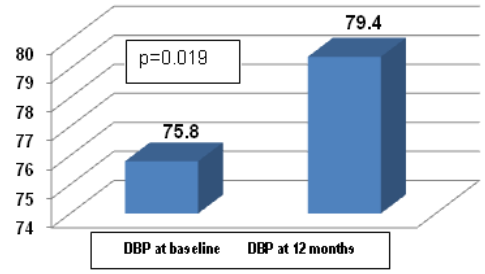

Fig.6. The comparison between DBP values at baseline (n=72) and at 12 months (n = 69). Wilcoxon test for paired values

At inclusion, hypertension was present in 43.1% (n=31) of all patients. Between the number of patients with hypertension present at baseline and at 12 months, there were significant differences; 4.9% of patients had higher blood pressure values at the end of chemotherapy (χ^2 test, p <0.001)(Figure 7).

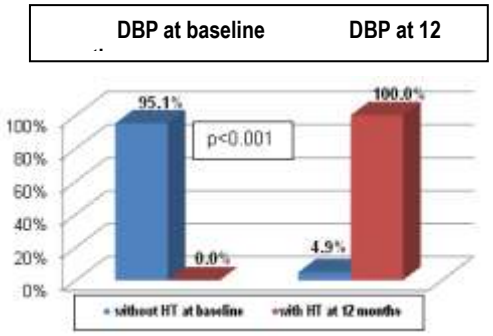

Fig.7. The comparison between the proportion of hypertension at baseline (n=72) and at 12 months (n = 69). χ^2 Test

There was a significant correlation, reverse, between systolic blood pressure and EF at 12 months (r = - 0.364, p = 0.002)(Figure 8).

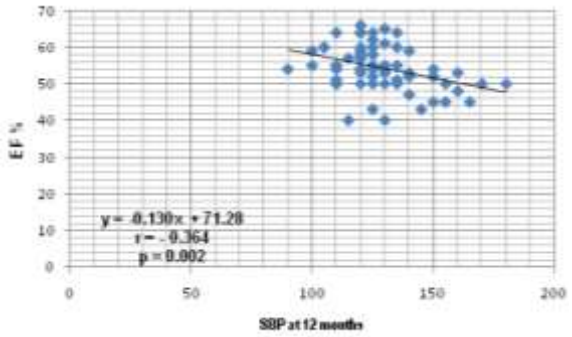

Fig.8. The correlation between systolic blood pressure and EF at 12 months (n = 69)

Diastolic blood pressure at 12 months was significant inverse and medium correlated with EF at 12 months (r = -0.400, p = 0.001)(Figure 9).

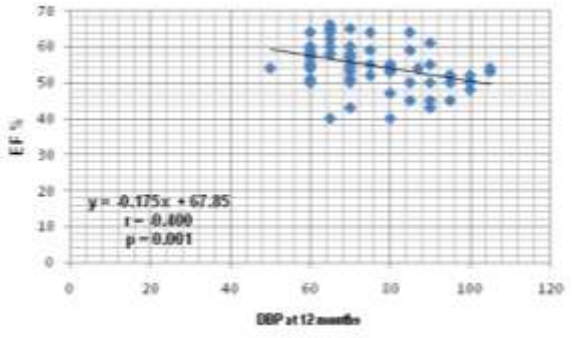

Fig. 9. The correlation between diastolic blood pressure and EF at 12 months (n = 69)

At first examination the metabolic syndrome (MS) was present in 33.3% of patients (n = 24). Obesity prevalence was of 37.5% (n = 27). Table XIII shows the percentage distribution of MS according to the Seidman

criteria for EF recommended by the ESMO 2012 guidlines. The differences were not significant, there was no association between the presence of MS and decreased EF ($\chi 2$ test, p = 0.387).

Table XIII. The percentage distribution of MS according to the Seidman criteria for EF

EF	Metabolic syndrome	
	No	Yes
EF>55%	**3**	**30**
	9.1%	90.9%
Decrease <5%	**1**	**16**
	5.9%	94.1%
Decrease between 5-10%	**2**	**6**
	25%	75%
Decrease >10%	**0**	**11**
	0%	100.0%

SCORE risk correlation at 12 moths with decreased EF according to the Seidman classification, showed a significant correlation between SCORE risk and decreased EF for the women who had at 12 months an EF less than 55% (χ^2 test, p = 0.001), is represented in Table XIV and Figure 10.

Table XIV. The percentage representation of the correlation between SCORE risk and decreased EF according to the Seidman criteria at 12 months (n = 69)

EF	SCORE risk			
	<5%	5-10%	10-15%	>15%
EF>55%	1	5	6	21
	3.0%	15.2%	18.2%	63.6%
Decrease <5%	1	7	1	1
	10.0%	66.7%	10.0%	10.0%
Decrease between 5-10%	1	8	1	2
	8.3%	70.0%	8.3%	16.7%
Decrease > 10%	1	8	4	1
	5.9%	64.7%	23.5%	5.9%

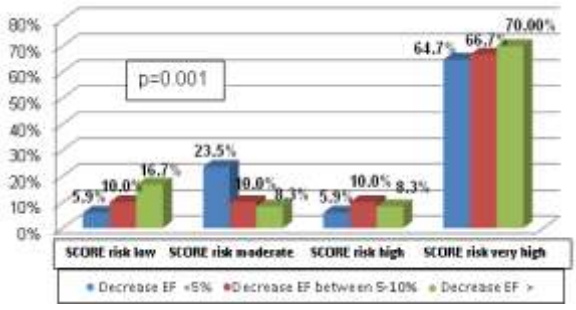

Fig.10. The graphical representation of the correlation between SCORE risk categories and EF according to the Seidman criteria at 12 months (n = 69). $\chi 2$ Test

DISCUSSION

In total, 72 women (mean age, 55.4±11.80 years) were enrolled. This study was conducted over a period of 12 months for each patient, from January, 2010, to November, 2014. The patients had different numbers of chemotherapy cycles with trastuzumab during the study. The inclusion criteria were: age > 18 years, normal sinus rhythm, LVEF > 50%, HER2 overexpression and trastuzumab therapy.

In this study were evaluated the cardiac performance expressed by left ventricular ejection fraction and diastolic function parameters (end-diastolic volume, IVRT and E/A ratio) in patients with HER-2 positive breast cancer under chemotherapy treatment with trastuzumab.

SCORE risk correlation at 12 moths with decreased EF, according to the Seidman classification (14) showed a significant correlation between SCORE risk and decreased EF for the women who had at 12 months an EF less than 55% (χ^2 test, p = 0.001).

This correlation explains the deaths of the 3 patients, two with the advent of cardiotoxicity after 6 months of enrollment, with a marked decrease in EF from 55% to 25% and from 60% to 25%, both overlapping risk factors.

The first patient died 3 months after enrollment, was 57 years old, with right breast cancer, presented the SCORE risk 21%, being hypertensive, dyslipidemic and obese with BMI 31.4 kg / m^2, total cholesterol = 290 mg/dl and triglycerides = 200 mg/dl.

The second patient died at 6 months from baseline, 65 years old, with left breast cancer classified T3N1M0, presented the SCORE risk 28%, with uncontrolled values of BP under antihypertensive therapy (HT=160/100 mmHg), dyslipidemia, obesity (BMI=33.22 kg/m^2), total cholesterol = 230 mg / dl and with lipid-lowering therapy.

The third death occurred suddenly in a 67 years old patient, with left breast cancer classified T2N0M0 and preserved EF at 52% at 6 months from baseline. The cause of death was probably malignant arrhythmia, because the previously 24 Hour Holter ECG performed documented recurrent episodes of sustained ventricular tachycardia. The patient had the SCORE risk 24%, being hypertensive, dyslipidemic and diabetic (BMI= 21.88 kg / m^2).

The entire risk spectrum gender-specific and pathophysiology of cardiac ischemia, extends beyond obstructive coronary disease to include other causes, such as microvascular dysfunction that is not included in the standard definition of coronary artery disease, which should include and other forms of ischemic heart disease.

The diagnosis and evolution is further challenged by sex/gender differences in variable sensitivity and presentation of cardiac biomarkers, imaging parameters, and risk SCORE (23).

The first two deaths confirm the observation of literature according to which women diagnosed with obesity and breast cancer have an increased risk of death. The relationship between obesity as a risk factor

and breast cancer is complex, having a protective role in young women and increase the risk to the elderly.

According to Biglia *et al* in a study on 2148 patients (592 premenopausal, 1556 postmenopausal), high BMI was significantly associated with larger size tumor both in pre ($p = 0.01$) and postmenopausal women ($p = 0.00$). Premenopausal women with obesity showed worse histopathologic features (presence of vascular invasion and more metastatic axillary lymphnodes) compared to under/normal weight women (24).

The metabolic syndrome is characterized by insulin resistance/hyperglycemia and subacute chronic inflammation, two conditions that associated is offering a plausible link with breast cancer. Thus, in addition to the increased risk of cardiovascular morbidity and mortality, women with MS is also in a high risk group of developing breast cancer, more often with poor prognosis (25).

Colonna *et al*. conducted a retrospective study on 860 patients with breast cancer to determine the relationship between the individual components of the metabolic syndrome and this type of cancer. The data showed the incidence of metabolic syndrome in 15% of women, 16% had elevated blood glucose levels, 26% were obese, 54% had hypertension and 30% had dyslipidemia. According to the study, presence of MS has not contributed to a more aggressive evolution of the disease (26). In the present study, the incidence of MS was 33.3%, 12.5% of patients were diabetic, 37.5% were obese and 43.1% hypertensive.

Unexpectedly, active smoking has not been proved so far to be a risk factor in breast cancer. However, existing data have raised serious doubts regarding passive smoking but also in this case, the evidence is still insufficiently clarified (27). In the present study, 19 patients was smoking (26.4%).

Imaging monitoring of the heart function is recommended during and after chemotherapy for early diagnosis of heart dysfunction (5). However, the validity of the 2-dimensional echocardiography in detecting cardiac dysfunction in this population has not been tested.

Thavendiranathan *et al*. observed in a study on 56 patients undergoing chemotherapy (all women), the best echocardiographic method for sequential quantification of EF and cardiac volumes. A temporal variability of 0.06 might occur in EF with noncontrast 3DE due to measurement variability and physiological differences, whereas this might be > 0.10 with 2D methods. 3DE also had the best intra- and inter-observer as well as test-retest variability (28).

3D Echocardiography is the one that determines most accurately left ventricular function, with the lowest rate of estimation, but this determination is not frequently available for clinical use (29).

Geisbergb *et al*. examined Tissue Doppler imaging techniques in studies with anthracycline cardiotoxicity. In small studies, these techniques which also investigates the strain, appear to be promising for the detection of early subclinical changes in cardiac performance and to anticipates a decrease in EF. Are not yet available long-term data performed on large populations of patients, to confirm the clinical relevance of these measurements (30).

CONCLUSIONS

- It is necessary to explore the cardiovascular system both clinical and paraclinical carefully before initiating chemotherapy which is known to be associated with significant cardiotoxicity.
- The presence of cardiovascular risk factors in oncology patients on cytostatic therapy requires special attention.
- The presence of an accumulation of risk factors prior to cytostatic therapy contributes to the emergence of cardiotoxicity, especially after cumulative doses of anthracyclines or combination of several chemotherapeutics.
- In this study, there was a significant correlation between the SCORE risk and decreased EF in patients who at 12 months had an EF less than 55%. Increased SCORE risk> 5% requires therapeutic and prophylactic interventions for prevention of cardiotoxicity.
- Echocardiography is the standard procedure for evaluating basal structure, performance and cardiac hemodynamic. LVEF assessment is mandatory to investigate cardiac function prior to initiation of treatment with potentially cardiotoxic.
- Observe the ESMO guidelines, where the necessity of the cardiac performance evaluation before treatment with cytostatic that can lead to a potential irreversible cardiotoxicity (type I) or reverse (type II) is class I of evidence and recommendation level A.
- Seidman criteria, recommended by ESMO indicate that 2D echocardiography for measure decrease of EF, represents an optimum solution for the evaluation of cardiotoxicity, although variability intra- and inter-observer limits its usefulness.
- Assessment of diastolic dysfunction revealed significant differences between the proportions of patients classified initially and finally with diastolic dysfunction at 12 months and may be an optimal criterion for tracking cardiotoxicity installation.
- 3D echocardiography assess the most accurate the LV function, with the lowest rate of estimate, but this measurement is not frequently available for clinical use.

REFERENCES

1. Schwartz ML. Cardio-oncology for the 21st century: introduction. *Seminars in oncology*. 2013;40(2):145-6.
2. Bovelli D, Plataniotis G, Roila F. Cardiotoxicity of chemotherapeutic agents and radiotherapy-related heart

disease: ESMO Clinical Practice Guidelines. *Annals of Oncology*. 2010; 21 Suppl 5: v277-82.
3. Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M, et al. Adjuvant trastuzumab in HER2-positive breast cancer. *The New England Journal of Medicine*. 2011;365(14):1273-83.
4. Suter TM, Ewer MS. Cancer drugs and the heart: importance and management. *European Heart Journal*. 2013;34(15):1102-11.
5. Curigliano G, Cardinale D, Suter T, Plataniotis G, de Azambuja E, Sandri MT, et al. Cardiovascular toxicity induced by chemotherapy, targeted agents and radiotherapy: ESMO Clinical Practice Guidelines. *Annals of Oncology*. 2012;23 Suppl 7:vii155-66.
6. Abu-Khalaf MM, Juneja V, Chung GG, DiGiovanna MP, Sipples R, McGurk M, et al. Long-term assessment of cardiac function after dose-dense and -intense sequential doxorubicin (A), paclitaxel (T), and cyclophosphamide (C) as adjuvant therapy for high risk breast cancer. *Breast Cancer Research and Treatment*. 2007;104(3):341-9.
7. Onitilo AA, Engel JM, Stankowski RV. Cardiovascular toxicity associated with adjuvant trastuzumab therapy: prevalence, patient characteristics, and risk factors. *Therapeutic Advances in Drug Safety*. 2014;5(4):154-66.
8. Albini A, Pennesi G, Donatelli F, Cammarota R, De Flora S, Noonan DM. Cardiotoxicity of anticancer drugs: the need for cardio-oncology and cardio-oncological prevention. *Journal of the National Cancer Institute*. 2010;102(1):14-25.
9. Cardinale D, Bacchiani G, Beggiato M, Colombo A, Cipolla CM. Strategies to prevent and treat cardiovascular risk in cancer patients. *Seminars in Oncology*. 2013;40(2):186-98.
10. Witteles RM, Fowler MB, Telli ML. Chemotherapy-associated cardiotoxicity: how often does it really occur and how can it be prevented? *Heart Failure Clinics*. 2011;7(3):333-44.
11. Pai VB, Nahata MC. Cardiotoxicity of chemotherapeutic agents: incidence, treatment and prevention. *Drug Safety*. 2000;22(4):263-302.
12. Jensen BV, Skovsgaard T, Nielsen SL. Functional monitoring of anthracycline cardiotoxicity: a prospective, blinded, long-term observational study of outcome in 120 patients. *Annals of Oncology*. 2002;13(5):699-709.
13. Aldiab A. Cardiotoxicity with adjuvant trastuzumab use in breast cancer: A single institution's experience. *Journal of the Saudi Heart Association*. 2010;22(3):133-6.
14. Seidman A, Hudis C, Pierri MK, Shak S, Paton V, Ashby M, et al. Cardiac dysfunction in the trastuzumab clinical trials experience. *Journal of Clinical Oncology*. 2002;20(5):1215-21.
15. Aitelhaj M, Lkhouyaali S, Rais G, Mohtaram A, Raissouni S, Ghissassi B, et al. Cardiac safety of the adjuvant Trastuzumab in a Moroccan population: observational monocentric study of about 100 patients. *BMC Research Notes*. 2013;6:339.
16. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, et al. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *Journal of Clinical Oncology*. 1998;16(8):2659-71.
17. Sheppard RJ, Berger J, Sebag IA. Cardiotoxicity of cancer therapeutics: current issues in screening, prevention, and therapy. *Frontiers in Pharmacology*. 2013;4:19.
18. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). *European Heart Journal*. 2012;33(13):1635-701.
19. Rossi G. [Diagnosis and classification of diabetes mellitus]. Recenti progressi in medicina. 2010;101(7-8):274-6.
20. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*. 2001;285(19):2486-97.
21. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren WM, et al. [European Guidelines on Cardiovascular Disease Prevention in Clinical Practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts)]. *Giornale italiano di cardiologia* (2006). 2013;14(5):328-92.
22. Arsenault BJ, Rana JS, Stroes ES, Despres JP, Shah PK, Kastelein JJ, et al. Beyond low-density lipoprotein cholesterol: respective contributions of non-high-density lipoprotein cholesterol levels, triglycerides, and the total cholesterol/high-density lipoprotein cholesterol ratio to coronary heart disease risk in apparently healthy men and women. *Journal of the American College of Cardiology*. 2009;55(1):35-41.
23. Safdar B, Nagurney JT, Anise A, DeVon HA, D'Onofrio G, Hess EP, et al. Gender-specific Research for Emergency Diagnosis and Management of Ischemic Heart Disease: Proceedings from the 2014 Academic Emergency Medicine Consensus Conference Cardiovascular Research Workgroup. *Academic Emergency Medicine*. 2014;21(12):1350-60.
24. Biglia N, Peano E, Sgandurra P, Moggio G, Pecchio S, Maggiorotto F, et al. Body mass index (BMI) and breast cancer: impact on tumor histopathologic features, cancer subtypes and recurrence rate in pre and postmenopausal women. *Gynecological Endocrinology*. 2013;29(3):263-7.
25. Hauner D, Hauner H. Metabolic syndrome and breast cancer: is there a link? *Breast Care* (Basel, Switzerland). 2014;9(4):277-81.
26. Colonna SV, Douglas Case L, Lawrence JA. A retrospective review of the metabolic syndrome in women diagnosed with breast cancer and correlation with estrogen receptor. *Breast Cancer Research and Treatment*. 2012;131(1):325-31.
27. Sadri G, Mahjub H. Passive or active smoking, which is more relevant to breast cancer. *Saudi Medical Journal*. 2007;28(2):254-8.
28. Thavendiranathan P, Grant AD, Negishi T, Plana JC, Popovic ZB, Marwick TH. Reproducibility of echocardiographic techniques for sequential assessment of left ventricular ejection fraction and volumes: application to patients undergoing cancer chemotherapy. *Journal of the American College of Cardiology*. 2013;61(1):77-84.
29. Armstrong GT, Plana JC, Zhang N, Srivastava D, Green DM, Ness KK, et al. Screening adult survivors of childhood cancer for cardiomyopathy: comparison of echocardiography and cardiac magnetic resonance imaging. *Journal of Clinical Oncology*. 2012;30(23):2876-84.

30. Geisberg CA, Sawyer DB. Mechanisms of anthracycline cardiotoxicity and strategies to decrease cardiac damage. *Current Hypertension Reports*. 2010;12(6):404-10.

EVALUAREA CARDIOTOXICITĂȚII INDUSĂ DE CHIMIOTERAPIE. CORELAȚII CU FACTORII DE RISC CARDIO-VASCULARI

REZUMAT

Scop: Identificarea factorilor de risc ce determina complicații cardiovasculare în timpul tratamentului chimioterapic poate ajuta la prevenirea și reducere toxicității cardiace. Acest studiul își propune evaluarea implicării factorilor de risc cardiovasculari în apariția cardiotoxicității la pacientele cu cancer de sân.

Materiale si metode: Un studiu prospectiv a fost efectuat pe 72 de femei cu cancer de sân HER2-pozitive. Criteriile de includere au fost: vârsta> 18 ani, ritm sinusal regulat, fracție de ejectie (FEVS)> 50%, HER2 pozitive și tratament cu trastuzumab. La toate pacientele au fost cuantificați factorii de risc cardiovasculari. Pentru evaluarea riscului SCORE, a fost utilizată diagrama pentru populaţiile cu risc înalt de mortalitate cardiovasculară. Sindromul metabolic a fost diagnosticat în conformitate cu criteriile Federației Internaționale de Diabet. Conform criteriilor Seidman și recomandărilor din ghidul ESMO 2012 pacientele au fost examinate la momentul inițial și la 3, 6 și 12 luni de intrarea în studiu, iar cardiotoxicitatea fiind definită ca scăderea FEVS.

Rezultate: Vârsta medie la momentul includeri în studiu a fost de 55.4 ± 11.80 ani. Hipertensiunea a fost prezentă la 43,1% (n = 31) din paciente și a crescut procentual cu 4,9% la 12 luni (test $\chi 2$, p <0,001). Analiza factorilor de risc cardiovasculari arată că sindromul metabolic a fost prezent la 33,3% dintre paciente (n = 24), 12,5% dintre paciente fiind diabetice iar 37,5% fiind obeze. Testul Spearman a relevat corelații semnificative între scăderea FEVS cu 5-10% și > 10%, și numărul total de cicluri chimioterapice. Nu s-a realizat o corelație semnificativă între risc SCORE și scăderea FEVS <55% la 12 luni (test $\chi 2$, p = 0,001). Cardiotoxicitatea severă a apărut la 3 paciente care au prezentat valori ale riscului SCORE foarte crescute.

Concluzii: Evaluarea factorilor de risc cardiovasculari implicați în cardiotoxicitatea indusă de trastuzumab și monitorizarea adecvată a pacientelor în timpul chimioterapiei permite utilizarea sigură și eficientă a acestui tratament adjuvant.

Cuvinte cheie: cardiotoxicitate, chimioterapie, trastuzumab, factori de risc, cancer de sân

THERAPEUTIC OPTIONS IN ALLERGIC RHINITIS

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ABSTRACT

Allergic rhinitis is a type I allergic disease of the nasal mucosa, characterized by paroxysmal repetitive sneezing, watery rhinorrhea, and nasal blockage. The symptoms are often ignored by patients and physicians, and most affected individuals do not report their complaints or seek treatments. In Europe, AR affects at least 21-23% of the population.

Rhinitis can be classified in infectious (acute and chronic) and non-infectious (allergic and non-allergic).

Treatment options are a very difficult to assess for the medical doctor because of the various options and because of the fact that allergic rhinitis is a very common disease with a tendency to reoccur.

The current paper aims to asses a clear classification of the allergic rhinitis and to point out the main treatment courses for the disease.

Key words: allergic rhinitis, treatment, rhinorrhea, nasal obstruction.

INTRODUCTION

Allergic rhinitis is a type I allergic disease of the nasal mucosa, characterized by paroxysmal repetitive sneezing, watery rhinorrhea, and nasal blockage. The symptoms are often ignored by patients and physicians, and most affected individuals do not report their complaints or seek treatments. In Europe, AR affects at least 21-23% of the population (1).

Allergic rhinitis is classified into perennial and seasonal. The term rhinitis is usually used to define nasal mucosal inflammation, which, from a histopatological point of view it is an exudative inflammation but suppurative and allergic inflammation are particularly common (4). Both are characterized by leakage of serum components from vessels, edema, cell infiltration, and hypersecretion.

CLASSIFICATION OF RHINITIS

Rhinitis can be basically classified as infectious and non-infectious, both types with different subdivisions (Table I):

Medicament rhinitis is usually caused by the long-term administration of a variety of pills like antidepressants, sympathomimetics, β-stimulatory antihypertensives, bronchodilators, contraceptive pills or vasodilatory antihypertensives but the most common cause is the overuse and overdose of sympathomimetic nose drops prescribed for nasal blockage.

There are numerous theories that try to explain the mechanism of appearance allergic rhinitis, but their mechanisms remain largely unknown (7). The most important ones are the ones based on IgE antibody production. In response to antigen entry into the mucous membrane, IgE antibodies are produced in the nasal mucosa and regional lymphatic tissues. This type of reaction has two phases: an early phase and a late phase seen at 6-10 hours after the exposure. Most causative antigens are inhalation antigens, such as Dermatophagoides (a major antigen in house dust), pollens (trees, grasses, and weeds), fungi, and pets (11).

Table I. Classification of rhinitis

Rhinitis							
Infectious		**Non-infectious**					
Acute	Chronic	Allergic	Non-allergic				
			Vasomotor rhinitis = symptomatically similar to allergic rhinitis, but it cannot be identified as an allergy by tests	Rhinitis with eosinophilia syndrome	Rhinorrhea type, including gustatory rhinitis	Congestive Type (medicament rhinitis- and psychogenic rhinitis, pregnant rhinopathy, hormonal rhinitis, and cold rhinitis)	Dry type

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COMMON SYMPTOMS

Table II. Common symptoms of rhinitis.

Sneezing	Watery Rhinorrhea	Nasal Mucosal Swelling
Caused by histamine irritation of the sensory nerve (trigeminal nerve) in the nasal mucosa, transmitted to the sneezing center of the medulla oblongata	Most rhinorrhea is secreted from the nasal glands; 10% sensory nerve irritation in the nasal mucosa => parasympathetic nerve excitement => sneezing reflex. Acetylcholine is released from, histamine acts directly on the nasal mucosal vessels to cause plasma leakage.	Caused by interstitial edema in the nasal mucosa, due to plasma leakage, and congestion of the nasal mucosal vessels The direct actions of chemical mediators, such as histamine, PAF, prostaglandin D2, kinin, and particularly leukotriene, are essential Continuous antigen irritation cause chronic lesions.

DIAGNOSIS

To establish the diagnosis of allergic rhinitis is required a series of tests. The most common are the nasal eosinophil staining in the nasal secretion and serum IgE antibody measurement. We can use o series of tests that are based on skin reactions or serum allergen-specific IgE antibody measurements. A nasal mucosa provocation test can be conducted for house dust and ragweed, but their assessments may be difficult. Rhinoscopy and x-ray examinations can be performed for differential diagnosis, but usually the definite diagnosis is clinical and it is made based on three symptoms: sneezing and nasal itch, watery rhinorrhea, and nasal blockade (9).

TREATMENT

Usually, allergic rhinitis is manageable when treated, but resists cure so the treatment is aimed at improving the quality of life, its aim being to alleviate symptoms and remove difficulties with everyday life (5).

The first rule in a successful treatment plan is educating the patient. The patient has to avoid the antigens, keep humidity at 50% and room temperature at 20-25°C, improve ventilation and clean rooms, avoid using textile sofa, carpet wherever possible, prevent pollen inhalation or avoid contact with pets.

There are also a few other new methods of treating the allergic rhinitis and among that are:

- Specific immunotherapy which is indicated for patients that are over 6 years old and the therapy should be administrated for at least 3 years and its therapeutic effects often continue for several years after discontinuation of administration (12);
- Sublingual immunotherapy (SLIT)-it is not used on a large scale; SLIT has been established as evidence-based treatment for AR. It was introduced in latest update of ARIA guidelines in 2010 with weak recommendation and moderate quality of evidence for patient sensitive to pollen and low quality of evidence for house dust mite according to the GRADE system. Beside its efficacy in reducing the symptoms and medication scores recorded in meta-analyses, the unique advantages of long-lasting and preventive actions are due to profound and persistent modifications in the immune system (2).
- Probiotics - Preliminary data exist providing beneficial results in using probiotics in the treatment of allergic rhinitis and probiotics could emerge as a novel, complementary treatment option for AR.

In some cases the surgical treatment is proved to be effective. Nasal blockage in allergic rhinitis is often caused by nasal deformities, such as deviated septum, hypertrophic rhinitis, and nasal polyps. In this case, perform corrective surgery of nasal cavity to improve nasal ventilation. The main purpose is to alleviate nasal blockage (8).

Sometimes complications can occur. The most common are acute and chronic sinusitis, eosinophilic sinusitis, allergic conjunctivitis, and sometimes even asthma can occur (4).

Pregnant women should be approached with caution because during pregnancy, congestive and allergic rhinitis often occurs, and symptoms are exacerbated. If medication is required after the fourth month of pregnancy, minimize the use of local agents, such as DSCG, nasal spray mast cell stabilizer, nasal spray antihistamine, and nasal topical steroids.

In children allergic rhinitis is common among male infants, often complicated by atopic dermatitis and sometimes it may heal spontaneously.

CONCLUSION

Allergic rhinitis is one of the most prevalent diseases in young people and is responsible for a significant impairment in quality of life but its control is usually far from satisfactory (3). Medications provide some relief, but improvement is only partial. Recent evidence from double-blind, placebo-controlled, randomized clinical trials suggests that the more severe the disease, the greater the treatment effect. Anti-IgE shots show better efficacy, but are still very expensive. Anti-allergic immunotherapy is promising but new, well designed, long-term clinical trials are needed. Most patients seeking treatment for allergic rhinitis are polysensitized, and allergen immunotherapy has proven efficacy. Avoidance procedures could lead to improvement if they are designed as multi-trigger, multi-component interventions (14).

Table III. Therapeutic agents for allergic rhinitis

Therapeutic agents			Effects	Contraindications
Alpha-sympathomimetics (vasoconstrictor nose drops)			- temporarily alleviate nasal blockage	Transsphenoidal Hypophysectomy High Blood Pressure Overactive Thyroid Gland, Diabetes
Mast cell stabilizer (local agents (eye drops an nasal spray) and oral agents)			- mild effects - 2-week prolonged administration is required. - amelioration rates are increased by continuous administration	Paradoxical bronchospasm Liver disease Kidney disease
Chemical mediator receptor antagonists	Histamine H1 receptor antagonists (antihistamine)	First-generation antihistamine	- often cause adverse effects(sleepiness, impaired performance, dry mouth) - have immediate effects on sneezing and watery rhinorrhea.	Patients with glaucoma, prostatic hyperplasia, and asthma because of their potent anticholinergic effects [6] They have less central nervous system depressant actions in children than in adults. Caution should be exercised for excitatory effects, such as convulsions.
		Second-generation antihistamine	- are effective to some extent for nasal blockage aside from sneezing and watery rhinorrhea - priority indications are mild to moderate sneezing and rhinorrhea type	Severe hepatic disorders
	Leukotriene receptor antagonists (antileukotrienes)		- potent relaxing effects on the vascular smooth muscles of the nasal mucosa - enhancing effects on vascular permeability - stimulating effects on eosinophil migration. -effective for nasal blockage. - their effects are increased by prolonged administration.	
	Prostaglandin D2 and thromboxane A2 receptor antagonist		- enhances vascular permeability in the nasal mucosa and suppresses eosinophil migration by blocking thromboxane receptors, and suppresses eosinophil migration by blocking CRTh2 - they have strong delayed effects on nasal blockage	Asthma attacks Major liver diseases
Th2 cytokine inhibitors			- IPD inhibits the production of Th2 cytokines, such as IL-4 and IL-5, in T lymphocytes to alleviate allergic inflammation	
Steroids		Nasal steroids	- strong local effects in small amounts, - are poorly absorbed and readily degraded - they have few systemic adverse effects - exert their effects within 1-3 days	
		Steroids for internal use	- only for intractable cases with severe nasal blockage and laryngopharynx symptoms uncontrollable with nasal spray steroids, - prednisolone (20-40 mg/day) can be administered for 4-7 days at the start of treatment	
Alpha-sympathomimetics			- act on the α-receptors of vascular smooth muscles to cause vasoconstriction and temporarily alleviate nasal mucosal swelling. -Long-term continuous administration causes medicament rhinitis	

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REFERENCES

1. Yu YI, Cho JS, Lee KH, Kim KH, Hong SM, Kim SW. Clinical statistical study on offending allergens of patients with allergic rhinitis: prevalence of multiple sensitization. *Korean J Otolaryngol-Head Neck Surg*. 2003;46:48-53.
2. Bahceciler NN, Galip N, Cobanoglu N. Multiallergen-specific immunotherapy in polysensitized patients: where are we? *Immunotherapy*. 2013;5:183-190.
3. Veling MC, Trevino RJ. The treatment of allergic rhinitis with immunotherapy: a review of 1,000 cases. *Ear Nose Throat J*. 2001;80:542-543.
4. Asher MI, Montefort S, Bjorksten B, et al. ISAAC Phase Three Study Group. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet*. 2006;368:733-43.
5. Schwindt CD, Settipane R. Allergic rhinitis (AR) is now estimated to affect some 1.4 billion people globally and continues to be on the rise. Editorial. *Am J Rhinol Allergy*. 2012;26(Suppl 1):S1
6. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 Update (in collaboration with the World Health Organization, GA2LEN and AllerGen). *Allergy*. 2008;63(Suppl 86):8-160.
7. Incorvaia C, Di Rienzo A, Celani C, Makri E, Frati F. Treating allergic rhinitis by sublingual immunotherapy: a review. *Ann Ist Super Sanita*. 2012;48(2):172-176.
8. Pichler CE, Helbling A, Pichler WJ. Three years of specific immunotherapy with house-dust-mite extracts in patients with rhinitis and asthma: significant improvement of allergen-specific parameters and of nonspecific bronchial hyperreactivity. *Allergy*. 2001;56:301-306.
9. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol*. 1998;102: 558-562.
10. Durham S, Birk AO, Andersen JS. Days with severe symptoms: an additional efficacy endpoint in immunotherapy trials. *Allergy*. 2011;11:120-123.
11. Xiao JZ, Kondo S, Yanagisawa N, Takahashi N, Odamaki T, Iwabuchi N, et al. Probiotics in the treatment of Japanese cedar pollinosis: A double-blind placebo-controlled trial. *Clin Exp Allergy*. 2006;36:1425-35.
12. Till SJ, Francis JN, Nouri-Aria K, et al. Mechanisms of immunotherapy. *J. Allergy Clin. Immunol*. 2004;113(6):1025-34.
13. Cox L, Nelson H, Lockey R. Allergen immunotherapy: A practical parameter third update. *J. Allergy Clin. Immunol.* 2001;1(suppl):S1-S55.
14. Shedden A. Impact of nasal congestion on quality of life and work productivity in allergic rhinitis. *Treat Respir. Med*. 2005;4(6):439-446.

OPTIUNI TERAPEUTICE IN RINITA ALERGICA

REZUMAT

Rinita alergica este o reactive alergica de tipul I a mucoasei nazale, caracterizata prin episoade de stranut repetitive, rinoree apoasa si obstructive nazala. Simptomatologia este adesea ignorata de pacienti si chiar si de medici , iar cel mai mult s,unt afectati indivizii ce aleg sa ignore simtomatologia si sa nu se prezinte la medic. In Europa rinita alergica afecteaza 21-23% din populatie.
Rinita se poate clasifica in infectioasa (acuta si cronica) si non-infectioasa (alergica si non-alergica).
Optiunile terapeutice sunt multiple si greu de ales deoarece rinita alergica este o boala foarte frecventa in populatie si are tendinta de a recadea.
Cuvinte cheie: rinita alergica, tratament, rinoree, obstructive nazala.

KERATINIZED MUCOSA – PREDICTIVE FACTOR FOR THE HEALTH OF PERI-IMPLANT TISSUES

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ABSTRACT

Due to the enhanced proprieties of the oral implants and advanced surgical techniques in the recent years, prosthetic treatments using dental implants are recommended and performed on large scale. In order to raise even more the predictability of this type of oral implants treatments, there must be evaluated and corrected their present complications.

The inflammatory complications were found with the highest frequency compared to the other two categories - surgical and prosthetic. Among local risk factors for this type of complication are implant surface characteristics, history of periodontal disease and decrease width of keratinized mucosa around implants.

The role of the keratinized mucosa played in the peri-implant health is an controversial subject in the literature due to the lack of consensus regarding the conclusions of the studies developed in time with the aim to assess the effects of the absence of this type of mucosa on the tissues around dental implants. The width of the keratinized mucosa considered sufficient to maintain the health of the peri-implant tissue is considered at least 2 mm – of which 1 mm should be attached mucosa.

On the other hand, there are authors that consider that as long as the oral hygiene is properly performed, there are not sign of peri-implant inflammation irrespective the width of the keratinized mucosa.

This review aimed to assess the correlation between the changes on the peri-implant tissues and the width of keratinized mucosa. The inconsistency of the results of these studies do not establish an consensus regarding this clinical aspect. The limits are given, among others, by the different study designs used and there is recommended onward research.

The recommendation is that the prognosis for the oral implants surrounded by a narrow keratinized mucosa is good but it is compulsory for oral hygiene procedures to be rigorous. In addition, in areas that are hard to be accessed for cleaning or in esthetic areas, there are recommended additional therapeutic procedures to offer sufficient quantity of keratinized mucosa

Keywords: periimplantitis, keratinized mucosa, soft tissue management

INTRODUCTION

Due to the enhanced proprieties of the oral implants and advanced surgical techniques in the recent years, prosthetic treatments using dental implants are recommended and performed on large scale. In order to raise even more the predictability of this type of oral implants treatments, there must be evaluated and corrected their present complications.

A retrospective study made at the Faculty of Dentistry of the Harvard University, Boston, USA, and published in 2003 aimed to identify different types of complication of the oral implants. These complications were classified in three groups: inflammatory, surgical and prosthetic. The inflammatory complications were found with the highest frequency compared to the other two categories – 10.2 % of the patients treated presenting specific signs (1).

Among the inflammatory complications, those localized on the peri-implant tissues and induced by the dental plaque is found with a prevalence ranging between 25% and 45% (2). According to the conclusions of the Peri-implantitis Workshop of Periodontology, held in 2008, based on the analyzed studies, the inflammation of the peri-implant tissues prevalence varies as it follows: peri-implant mucositis – 80% and peri-implantitis – 28-53% of the total number of the patients with dental implants (3,4).

Inflammation localized on the tissues around implants that have been successfully osseointedrated, is caused by the microbial colonization and multiplication to which human body give an immune response. During the first phase, the inflammation affects only the soft tissue – pathology known as peri-implant mucositis, and then as a consequence of the evolution of the pathological process in absence of any therapeutic procedure, the inflammation extends to the hard tissue, causing the peri-implantitis (5-7).

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Peri-implant mucositis is defined as the reversible inflammation of the soft tissue around the implant, which is diagnosed by the following signs: bleeding on probing, increased depth of the peri-impant sulcus (4-5 mm) and no bone alteration (5,6,8,9). These clinical signs develop as a consequence of the inflammatory response of the host tissue that release cytokines, in a similar way as noticed in natural dentition tissues (10,11). In presence of a persistent local microbial colonization, the level of these cytokines significantly increases which has destructive effects on host tissues. The first destroyed is collagen fibers and next – the bone [7]. In this phase, the pathology has turned to peri-implantitis which is recognized by bleeding on probing, peri-implant pockets deeper than 5 mm, and the most important – peri-implant bone resorption (3,5-8,10).

Microbial colonization is developed because of the persistent dental plaque as a consequence of the unproper oral hygiene. Thus, dental implants inserted in areas hard to be cleaned are prone to development of peri-implantitis. Among local risk factors for this type of complication are implant surface characteristics, history of periodontal disease and decrease width of keratinized mucosa around implants (7).

Peri-implant keratinized mucosa is represented by keratinized epithelium that covers a dense connective tissue with no elastic fibers, that lays between free gingival margin and muco-gingival junction with a free component – external wall of the peri-implant sulcus/pocket, and an attached component – firmly connected to the periosteum. The width varies dependent to the location in the dental arch, the frenulum insertion and patient age (12-14). When it comes to edentulous areas, the width of the keratinized mucosa decreases as a consequence of the bone resorption which also leads to higher insertion of the mobile mucosa. Mobile mucosa has an increased number of elastic fibers and is connected to the peri-oral muscles and thus could cause traction of the soft tissue around the implant during the muscular activity (12,13).

The role of the keratinized mucosa played in the peri-implant health is an controversial subject in the literature due to the lack of consensus regarding the conclusions of the studies developed in time with the aim to assess the effects of the absence of this type of mucosa on the tissues around dental implants (12,14-20).

In the literature, the authors that support the importance of the presence of keratnized mucosa, use the following reasons (14-16,18-21):

- the traction forces caused by the mobile mucosa generate the movement of the external wall of the peri-implant sulcus during the mastication, thus accumulation of the dental plaque subgingivally
- the ease in oral hygiene procedures, decreasing the trauma and pain caused by the dental cleaning in areas where the bone resorption is advanced and the position of tongue, lips and chicks impede the access for the toothbrush
- mobile mucosa is unproper to form a good quality peri-implant sealing
- moreover, frequent traction caused by the mobile mucosa will jeopardize the peri-implant sealing
- the movement of the mobile mucoasa around implants could cause soft tissue recession and dental exposure
- peri-implant mucosa could collapse during the attachment/detachment of the prosthetic accessories

The width of the keratinized mucosa considered sufficient to maintain the health of the peri-implant tissue is considered at least 2 mm – of which 1 mm should be attached mucosa (19,20).

On the other hand, there are authors that consider that as long as the oral hygiene is properly performed, there are not sign of peri-implant inflammation irrespective the width of the keratinized mucosa (9,14,19,20).

RESULTS

The studies that aimed to assess the correlation between the changes on the peri-implant tissues and the width of keratinized mucosa showed contradictory results.

Analyzing the articles with this focus published till the present accessible on the PubMed database, we collected the results and distributed them into to major groups: supporting and denying the necessity of the keratinized mucosa for the peri-implant tissues health. We compared them using as parameters: efficacy of the oral hygiene, inflammation of the soft tissue, tissue recession, bone resorption and implant loss.

A study (8) developed in 2006 in Kristianstad – Sweeden, on 218 patients restored using 999 two stage oral implants, had a following time of 9-14 years. All the subjects enrolled in the study received oral hygiene instructions. Results revealed that plaque index, pocket depth and also quantity of pus increased with the decreasing of the width of the keratinized mucosa (using one or multi variable tests).

A study (22) published in 2006, developed in Michigan – USA on 69 patients restored using a total number of 339 dental implants, aimed to assess the influence of the keratinizemucosa and examined in addition to other studies the width of attached mucosa. Results obtained showed that plaque index and gingival index had significant higher values in peri-implant sits with keratinized mucosa narrower than 2 mm and attached mucosa narrower than 1 mm. Yet, regarding the annual bone resorption, no significant differences were noticed depending on the quantity of keratinized mucosa.

A study (18) developed in 2003-2007 in Seoul – South Korea, on a sample of 100 patients who were inserted 273 two-stage oral implants, monitored the

evolution on an average period of 13 months (6-26 months). Restored areas were posterior areas (premolar and molar, maxillary and mandibular areas). Depending on the quantity of the keratinized mucosa, there were 186 implants withing sufficient category and 90 – insufficient. The results regarding the oral hygiene efficacy (plaque index), inflammatory signs (bleeding on probing, pocket depth) did not show significant differences between the two categories. However, regarding the soft tissue recession and the bone resorption, implants with insufficient keratinized mucosa being significantly more affected.

A retrospective study (12) published in 2008 and developed in Haifa-Israel on 32 patients who were restaured using single or multi unit fixed restorations on 63 implants. Results established a negative correlation between tissue recession and reduction of the level of peri-implant tissue ring and the width of the keratinized mucosa. Surprisingly, a positive correlation was determined between the width of keratinized mucosa and the pocket depth but the researchers consider the decrease of sulcus depth a consequence if tissue recession. In this study, the inflammation was analyzed also using test to reveal the level of inflammation markers and the results showed that the PgE2 had a significant increase in cases with insufficient keratinized mucosa.

In 2008 there have been published a cross-sectional study (23) applied in Cleveland – USA on 76 patients restored with fixed dentures on 200 oral implants. The researcher found significant higher values for plaque index, gingival index, bleeding on probing index for sites with less than 2 mm of keratinized mucosa than those wider than that. In addition, significant more bone resorption (assessed using radiographs) and regarding this parameter, the results remained unchanged irrespective the variables used in the analysis.

A multicentric study (19) (applied in 5 dental schools in USA and an private dental office in Great Britain) published in 2009, included 73 patients with completely edentulous arches who were restored using a total number of 386 stage two dental implants. They were monitored for 5 years, and only 58 patients with 307 implants were maintained in the study until the final point. All the patients were given proper oral hygiene instructions. Results showed that plaque index and bleeding on probing index were correlated with the width of keratinize mucosa on lingual areas but no significant differences were obtained for buccal areas. Again, tissue recession and width of keratinized mucosa were found in negative correlation. Based on these results, the researchers determined the quantity of keratinized mucosa necessary for the maintenance of the peri-implant tissue health: 1-2 mm for efficient oral hygiene, 0-1 mm for absence of the inflammation, 1-2 mm for resistance to soft tissue recessions.

A cross-sectional study (20) published in 2009, applied in Isfahan – Iran, examined 27 patients with completely edentulous arches and restored using a total number of 66 oral implants. Patients enrolled were distributed into two groups: with a width keratinized mucosa higher or less than 2 mm. Results revealed significant differences between the two groups, with higher plaque index, gingival index, bleeding on probing index at patient in the second group. The same negative correlation was found also for the tissue recession parameter. Still, no significant differences were found for the pocket depth. Also, although the values of the bone resorption were higher in sites with less than 2 mm of keratinized mucosa, the differences between groups were not statistically significant.

A longitudinal study (24) published in 2013, applied in Istanbul – Turkey, included 15 completely edentulous arch patients using a total number of 60 implants who were not only clinically and but also biochemically assessed and followed up for 12 months, taking into consideration for examination the quantity of keratinized mucosa. Plaque index and gingival index had significant higher values at sites with narrower than 2 mm of keratinized mucosa. Moreover, when it comes to inflammation markers assessed after harvesting sulcular fluid, the results revealed that TNF alpha value were significantly higher at this group, and the values increased at 12 months after the first assessment.

A study (25) published in 1994 was developed in Goteborg – Sweeden on 39 completely or partially edentulous arch patients who were restored on 171 oral implants. The results show that there are no significant changes in clinical signs at patients who do not benefit of a sufficient keratinized mucosa compared to those patients who have an adequate quantity of this type of mucosa. Plaque index and bleeding on probing index did not reveal significant differences. Thus, the authors do not support the concept of the necessity for a width of 2 mm of keratinized mucosa around oral implants.

Another study (26) published in 2012 developed in Sao Paolo – Brasil, in 109 patients with palatal cleft who were restored using 202 two stage dental implants and followed up for 1 year after the implant insertion. The subjects were divided in two groups depending on the width of keratinized mucosa: more or less that 2 mm. Only the buccal sites were examined this time. The results obtained revealed that plaque index and gingival index did not have significant differences between the two groups. Moreover, the bigger the width of the keratinized mucosa, the deeper the peri-implant pockets. Conclusion of this study on special category of patients was that plaque accumulation around the dental implants next to the cleft area is not influence of the keratinized mucosa.

CONCLUSION

The inconsistency of the results of these studies does not establish a consensus regarding this clinical aspect. The limits are given, among others, by the different study designs used and there are recommended onward research.

The conclusion and recommendation of the 6th European Workshop on Periodontology focused on Peri-implantitis are that the prognosis for the oral implants surrounded by a narrow keratinized mucosa is good but it is compulsory for oral hygiene procedures to be rigorous. In addition, in areas which are hard to be accessed for cleaning or in esthetic areas, there are recommended additional therapeutic procedures to offer sufficient quantity of keratinized mucosa (14).

REFERENCES

- McDermott NE, Chuang SK, Woo VV, Dodson TB. Coplications of dental implants: identification, frequency, and associated risk factors. *Int J Oral Maxillofac Implants*. 2003;18(6):848-855.
- Lindhe J, Karring T, Lang NP - Clinical periodontology and implant dentistry. Oxford: Blackwell Munksgaard, 2011;534.
- Lindhe J, Meyle J. Peri-implant diseases:Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol*. 2008;35 (Suppl. 8): 282-285.
- Salvi GE, Aglietta M, Eick S, Sculean A, Lang NP, Ramseier CA. Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans. *Clin. Oral Impl. Res.* 2012; 23: 182–190.
- Ata-Ali J, Flichy-Fernández AJ, Ata-Ali F, Peńarrocha-Diago M, Peńarrocha-Diago M - Clinical, Microbiologic, and Host Response Characteristics in Patients with Peri-implant Mucositis. *J Oral Maxillofac Implants.* 2013;23(8):883-890.
- Heitz-Mayfield LJA. Diagnosis and management of peri-implant diseases. *Australian Dental Journal*. 2008; 53(1 Suppl): 43-48.
- Nguyen-Hieu1 T, Borghetti A, Aboudharam G. Peri-implantitis: from diagnosis to therapeutics. *J Investig Clin Dent.* 2012; 3:79-94.
- Roos-Jansaker AM, Renvert H, Lindahl Ch, Renvert S. Nine-to fourteen-year followup of implant treatment. Part III: factors associated with peri-implant lesions. *J Clin Periodontol*. 2006; 33: 296-301.
- Heitz-Mayfield LJA. Peri-implant diseases: diagnosis and risk indicators. *J Clin Periodontol*. 2008; 35 (Suppl. 8):292-304.
- Eriesson I, Persson LG, Berglundh T, Marinello CP, Lindhe J, Klinge B. Different types of infiammatory reactions in peri-implant soft tissues. *J Clin Periodontol*. 1995; 22:255-261.
- Kozlovsky A, Tal H, Laufer BZ, Leshem R, Rohrer MD, Weinreb M, Artzi Z. Impact of implant overloading on the peri-implant bone in inflamed and noninflamed peri-implant mucosa. *Clin. Oral Impl. Res*. 2007; 18:601-610.
- Zigdon H, Machtei EE. The dimensions of keratinized mucosa around implants affect clinical and immunological parameters. *Clin. Oral Impl. Res.* 2008; 19:387-392.
- Wennstrom JL, Derks J. Is there a need for keratinized mucosa around implants to maintain health and tissue stability? *Clin. Oral Implants Res.* 2012; 23 (Suppl. 6):136-146.
- Basegmez C, Ersanli S, Demirel K, Bölükbasi N, Yalcin S. The comparison of two techniques to increase the amount of peri-implant attached mucosa: free gingival grafts versus vestibuloplasty.One-year results from a randomised controlled trial. *Eur J Oral Implantol.* 2012; 5(2):139-45.
- Mehta P, Peng LL. The width of the attached gingiva-Much ado about nothing? *J Dent.* 2010; 38:517-525.
- Park JC, Yang KB, Choi Y, Kim YT, Jung W, Kim CS, Cho KS, Chai JK, Kim CK, Choi SH. A simple approach to preserve keratinized mucosa around implants using a pre-fabricated implantretained stent: a report of two cases. *J Periodontal Implant Sci.* 2010; 40:194-200.
- Lorenzo R, Garcı´a V , Orsini M, Martin C, Sanz M. Clinical efficacy of a xenogeneic collagen matrix in augmenting keratinized mucosa around implants: a randomized controlled prospective clinical trial. *Clin. Oral Impl. Res.* 2012; 23:316-324.
- Kim BS, Kim YK, Yun PY, Yi YJ, Lee HJ, Kim SG, Son JS. Evaluation of peri-implant tissue response according to the presence of keratinized mucosa. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009; 107(3):24-8.
- Schrott AR, Jimenez M, Hwang JW, Fiorellini J, Weber HP. Five-year evaluation of the influence of keratinized mucosa on peri-implant soft-tissue health and stability around implants supporting full-arch mandibular fixed prostheses. *Clin. Oral Impl. Res.* 2009; 20:1170-77.
- Adibrad M, DDS, Shahabuei M, Sahabi M. Significance of the width of kertinized mucosa on the health status of the supporting tissue around implants supporting overdentures. *J Oral Implantol.* 2009; 5(5):232-7.
- Krygier G, Glick PL, Versman KJ, Dahlin CJ, Cochran DL. To minimize complications, is it essential that implant abutments be surrounded by keratinized tissue? *Int J Oral Maxillofac Implants.* 1997; 12(1):127.
- Chung DM, Oh TJ, Shotwell JL, Misch CE, Wang HL. Significance of keratinized mucosa in maintenance of dental implants with different surfaces. *J Periodontol.* 2006; 77(8):1410-20.
- Bouri A Jr, Bissada N, Al-Zahrani MS, Faddoul F, Nouneh I. Width of keratinized gingiva and the health status of the supporting tissues around dentalimplants. *Int J Oral Maxillofac Implants*. 2008; 23(2):323-6.
- Boynueğri D, Nemli SK, Kasko YA. Significance of keratinized mucosa around dental implants: a prospective comparative study. *Clin Oral Implants Res.* 2013; 24(8):928-33.
- Wennström JL, Bengazi F, Lekholm U. The influence of the masticatory mucosa on the peri-implant soft tissue condition. *Clin Oral Implants Res.* 1994; 5(1):1-8.
- Esper LA, Ferreira SB Jr, de Oliveira Fortes Kaizer R, de Almeida AL. The role of keratinized mucosa in peri-implant health. *Cleft Palate Craniofac J.* 2012; 49(2):167-70.

MUCOASA KERATINIZATĂ – FACTOR DE PREDICȚIE PENTRU SĂNĂTATEA ȚESUTURILOR PERI-IMPLANTARE

REZUMAT

Datorită îmbunătățirii proprietăților implanturilor orale și tehnicilor chirurgicale avansate în ultimii ani, restaurarea protetică pe implanturi sunt recomandate și utilizate pe scară largă. Pentru creșterea predictibilității acestui tip de tratament, este necesar să fie evaluate și minimalizate potențialele complicații.

Complicațiile inflamatorii ale tratamentului prin implanturi dentare a fost întâlnit cu frecvența cea mai mare, prin comparație cu celelalte două tipuri de complicații asociate tratamentului implantar: chirurgicale și protetice. Printre factorii de risc locali se regăsesc caracteristicile de suprafață, istoricul de boală parodontală și lățimea scăzută a mucoasei keratinizate periimplantare.

Rolul mucoasei keratinizate din jurul implanturilor în statusul țesuturilor periimplantare este un subiect controversat în literature, datorită lipsei unei consecvențe în ceea ce privește concluziile studiilor efectuate, care au urmărit evaluarea consecințelor pe care le are lipsa mucoasei keratinizate atașate de os în jurul implanturilor orale. Lățimea mucoasei keratinizate, considerate a fi suficientă pentru menținerea sănătății țesuturilor periimplanare, este de minim 2 mm, din care 1 mm trebuie să fie mucoasă atașată.

Pe de altă parte, sunt autori care consideră că dacă igiena orală este realizată adecvat, nu apar semne de inflamație periimplantară, indiferent de lățimea mucoasei keratinizate.

Review-ul de față are ca scop evaluarea corelației dintre modificările la nivelul țesuturilor periimplantare și lățimea mucoasei keratinizate. Rezultatele studiilor analizate nu a dus la un consens în ceea ce privește acest aspect clinic. Limitele s-au datorat designului diferit al acestor studii, de unde necesitatea unor studii suplimentare.

Recomandarea este ca, pentru un prognostic bun al implanturilor dentare care prezintă o mucoasă keratinizată îngustă, igiena orală este obligatoriu să fie riguroasă. Totuși, se recomandă ca, în zonele în care accesul este îngreunat pentru igienizare sau în zonele frontale ce implică cerințe estetice, să fie realizate intervenții de augmentare a mucoasei keratinizate.

Cuvinte cheie: periimplantită, mucoasă keratinizată, management țesut moale

LEUKOTRIENES AND PAIN-NEW THERAPEUTIC PERSPECTIVES

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ABSTRACT
Leukotrienes (LTs) are an important eicosanoids group involved in the mechanism of inflammatory and non-inflammatory pain. The involvement of LTs in the peripheral mechanism of nociception is supported by data which show that many inflammation mediators (TNF, some interleukines and others) stimulate LTB_4 and peptidoleukotrienes synthesis. LTB_4 antagonists and 5-lipoxygenase inhibitors decrease the peripheral inflammatory nociception. LTs are also involved in the neurophatic pain. The activation of $cysLT_2$ receptor from dorsal root neurons can modulate $TRPV_1$ receptor activity. Our experimental data show that montelukast ($cysLT_1$ receptor antagonist) increased the mechanical nociceptive threshold in rats. The inhibition of LTs synthesis, as well as BLT_1, BLT_2, $cysLT_1$ and $cysLT_2$ receptors blockade could be in the future a new way in pain therapy.
Key words: leukotrienes,inflammarory pain, neuropatic pain, 5-LOX inhibitors, montelukast, risedronate, licofelone

GENERAL CONSIDERATIONS

This group of active lipids are an important part of eicosanoids derived by arachidonic and eicosapentaenoic acids under the influence of 5-lipooxygenase (5-LOX).The LTs are oxygenated metabolites of arachidonic and eicosapentaenoic acids with an important biological activity and a lot of involvements in normal functions of the cells but also in the pathogenesis of various diseases (1).LTs synthesis is present in different cells and tissues from human and animal body The LTs derived from arachidonic acid are the most important group of leukotrienes. The arachidonic acid is released from membrane phospholipids by phospholipase A2. The first step in the synthesis of LTs is the transformation of arachidonic acid into LTA4. This is an inactive compound but is rapidly metabolized in two important groups of LTs: i) LTB_4 (which doesn't contain in its molecule aminoacids rests); ii) Peptidoleukotrienes LTC_4, LTD_4, LTE_4 (which have an aminoacid sequence in the molecule: peptide-containing LTs)

The LTA4 is metabolized to LTC4 by LTC synthase. The LTC4 is metabolized in LTD4 by a gamma-glutamyltransferase and by gamma-glutamilleukotienase (2). The metabolism of LTD4 in LTE4 is produced for about 40% by a dipeptidase but now is not clear what is the enzyme involved for other 60% from LTE4 synthesis (3).

All of the leukotrienes above mentioned are derived from arachidonic acid. There are also leukotrienes which derive from eicosapentaenoic acid (EPA). For example, LTB_5 derived from EPA was shown to be approximately 30 times less active than LTB_4 (derived from arachidonic acid). The potency of LTB_5 in potentiating bradikinine-induced plasma exudation was ten times lower than that of LTB_4 (4).

Besides the most active leukotriene-producing cells are the leukocytes, but also lung, kidney and liver parenchyma which actively synthesize these eicosanoids. In leukotriene's case, an important role plays also the transcelullar synthesis. Although endothelial cells can't generate LTs from arachidonic acid it has been shown that polymorphonuclear leukocytes can transfer to endothelial cells LTA_4 (an intermediary compound) as substrate for peptidoleukotrienes synthesis. All leukotrienes act on membrane receptors. There are four types of membrane G-protein coupled receptors for LTs.BLT1 and BLT2 are receptors for LTB_4 and LTB_5 and $cysLT_1$ and $cysLT_2$ are receptors for peptidoleukotrienes (5,6).

The most known involvements of the leukotrienes in different pathogenic processes are those from bronchial asthma, allergic rhinitis, atherosclerotic lesions, idiopathic pulmonary fibrosis, inflammation, anaphylaxia, but also in psoriasis, ulcerative colitis,nasal polyposis, chronic urticaria, allergic conjunctivitis, migraine, atopic dermatitis and reumathoid arthritis pathogeny (7-9).

LTs are involved in inflammatory and non-inflammatory pain. LTs are eicosanoids with a pro-inflammatory profile involved in pathogenesis of several types of inflammation (10,11). Several studies have demonstrated that LTs are involved in pathogenesis of inflammatory pain.

Cysteinyl-LTs are potent inflammatory mediators but are involved also in central nociception and peripheral nociception.

There are clinical and experimental data which show the involvement of LTs in nociception and pain.

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Inflammatory pain

Several studies have demonstrated that LTs are involved in pathogenesis of inflammatory pain. There are data that showed that leukotrienes are a part of the active pro-nociceptive factors. Previous studies reported that some lipoxygenase metabolites are involved in hyperalgesia in peripheral inflammation. In these cases, LTs were released from infiltrated immune cells, such as neutrophils, and may have an effect on nociceptors in peripheral inflamed tissues. The blocking agents of the both LTB4 receptors and cys- LT1 and cys-LT2 receptors influence the nociception (12,13).

Several studies have demonstrated that LTs are involved in pathogenesis of inflammatory pain.

Intraplantar LTs receptor agonist administration induces a powerful hyperalgesic response and decreases the mechanical and thermal pain threshold of C-fibers nociceptors (14). The administration of a BLT receptor antagonist suppresses this hyperalgesia and also blocks the painful response after experimental carrageenin administration in rats (15).

There are data that showed that leukotrienes are a part of the active pro-nociceptive factors.The blocking agents of the both LTB_4 receptors and cys- LT_1 and cys-LT_2 receptors influence the nociception.

Zafirlukast (a cysLT_1 antagonist) 2.5-20 mg/kg per os produce a significant dose-dependent antinociceptive effect against the experimental acid-acetic induced chemonociception in mice and attenuated the carrageenan-induced peripheral hyperalgesia, but the same antagonist of cysLT_1 receptors does not change the pain threshold in central nociception (11).

LTB_4 which mediates different inflammatory processes is involved also in the mechanism of hypernociception and inflammatory pain. Intraarticular experimental administration of LTB_4 by joint injection induced a dose dependent hypernociception (13). A part of hypernociception induced by intrajoint LTB_4 administration of zymosan was reduced by celecoxib (COX-2 inhibitor) or indomethacin 5mg/kg. This fact shows that LTB_4 effect in nociception is partially mediated by the increased prostaglandin's synthesis.

The sensitization of nociceptors is an essential phenomenon of inflammatory pain. The up-regulation of nociceptors is involved in hyperalgesia.LTB_4 participates at the genesis of inflammatory hyperalgesia (16). In the pain produced in the rat paw subsequent to the intraplantar injection with ovalbumin the level of interleukines IL-1ß and IL-8 but also TNFα is higher. TNFα and some interleukines stimulate the LTB_4 synthesis. The administration of MK886 (an LTB_4 synthesis inhibitor) and a LTB_4 selective receptor antagonist CP105696 reduced LTB_4 mediated ovalbumin induced hypersensibility. The main source for LTB_4 synthesis in these situations are the polymorphonuclear neutrophils. A very selective non-redox 5-LOX inhibitor, PL-4191834 is very efficient in reduction of acute experimental inflammation and pain (17).

The endothelin-1(ET-1) involved in inflammatory events including pain, stimulates the LTB_4 synthesis, BQ-123 and BQ-788 (two endothelin receptors antagonists) decreased the LTB_4 level in a murine model of zymosan-induced arthritis (18). Risedronate has an important anti-inflammatory and anti-nociceptive activity in rats receiving zymosan intraarticulary. This effect was associated to a reduction of LTB_4 joint synthesis (19). Risedronate action is not mediated or by endogenous opioid synthesis. The interleukins involved in the inflammation modulate cys-LT receptors expression at the level of cell membrane. The IL-4 increases the cell surface expression for cys-LT1 and cys-LT2 Interferon receptors (IFN gamma) stimulate cys-LT1, cys-LT2 and BLT1 receptor mRNA production and also the surface expression (20). Il-5 increases only cys-LT1 receptor surface expression (21).

The same involvement of LTB4 in pain mechanism exists also in carrageenan and LPS-induced inflammation. The ratio between different groups of eicosanoids plays an important role in roles in physiologic and pathophysiologic processes.

New groups of lipid mediators stimulate the resolution of acute inflammation. These include the resolvins (E-series and D-series), protectins (neuroprotectin D1/protectin D1), and maresins biosynthesized from omega-3 essential fatty acids.

The existing data involve more the leukotrienes in peripheral nociception. Others eicosanoids synthesized from arachidonic acid-like lipoxins are involved in the modulation of pain intensity. Chronic morphine experimental administration induced the development of hyperalgesia and the expression of spinal anti-nociceptive tolerance to morphine. LXA4ME, an analogue of LXA4 (lipoxin A4) treatment significantly attenuated the development of the expression of spinal antinociceptive tolerance to morphine in both mechanical and thermal experimental induced pain in rats. LXA4ME inhibited the activation of microglia and astrocytes, and reduced the expression of pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6, and tumour necrosis factor-α (TNF-α). At this moment, it is unclear which is the relationship between leukotrienes and resolvins in the field of nociception. The lipids mediators derived from ω-3 fatty acids such as docosahexaenoic acid and eicosapentaenoic acid called resolvins and protectins (22) which displayed anti-inflammatory actions could be also important for nociception (23). Docosahexaenoic acid (DHA) (22:6) which generates a part of resolvins and protectins is abundant in the brain and plays a neuroprotector role at this level (24).

Resolvins control the evolution of the inflammatory process and protect against the oxidative stress in experimental induced inflammation (25).

Transient receptor potential subtype vanilloid 1 (TRPV1) and TRP ankyryn 1 (TRPA1) are important for the inflammatory pain genesis via both peripheral mechanisms and spinal cord mechanisms (central sensitization). The resolvins are potent endogenous inhibitors for TRPV1/TRPA1 and inflammatory pain (26). The ratio between pro-inflammatory leukotrienes and anti-inflammatory (pro-resolving) lipids is important not only for the development of inflammation, but also in the modulation of the hypernociception. Resolvins reduced inflammatory pain via central and peripheral actions (27).

It is possible that the ratio between leukotrienes and prostaglandins as enhancing hypernociception eicosanoids and other eicosanoids as lipoxins, resolvins and protectins play a role in the modulation of nociception both at the central and peripheral level in our body.

Neuropathic pain

The neurophatic pain is produced by nerve injury or by diseases which damage the nerves. In the neuropathic pain, the peripheral nerve terminals sensitization is an important step. The molecules resulted after tissue injury act as pro-nociceptive mediators and produce peripheral nerve sensitization. Many types of trauma increased the tissues and the neutrophile leukotrienes synthesis. The level of cys- LTs increased in the dorsal spinal horn after peripheral nerve injury. The expression in CysLT1 mRNA in the spinal cord increased in the dorsal horn after peripheral nerve trauma. The CysLT2 mRNA was expressed in the white matter of the spinal cord, and this expression was not changed after nerve injury (28).

In the neuropathic pain, the peripheral nerve terminals sensitization is an important step. The molecules resulted after tissue injury act as pro-nociceptive mediators and produce peripheral nerve sensitization. Eicosanoids play important roles at the level of the central and peripheral nervous system. Many types of trauma increased the LTs synthesis in many tissues and in leukocytes. Leukotrienes are produced in nervous system so in neurons as well as in nevroglia. The LTs synthesis is mainly regulated by the activity of phospholipase A2 (which release arachidonic acid from phospholipids) and 5-LOX (29).

We tested the influence of montelukast (MK), a $cysLT_1$ antagonist in mechano-nociception in rats. It was tested the MK effect for 10mg/kg p. o.

Our data (30) showed that the association of MK with morphine significantly increased the threshold of mechanoalgesia compared to the group which received only morphine. These data are consistent with the idea of peptidoleukotriene involvement in mechano-nociceptive mechanism.

In this way, the research showed that MK can influence the morphine action. The results show that MK raised the mechanical nociceptive threshold in rats which received morphine. These data suggest that is possible that montelukast facilitates the morphine action on μ receptors or that $cysLT_1$ receptors are involved in reduction of morphine analgesic effect after stimulation of μ receptors. The increase of LT synthesis in spinal microglia produced via p38 mitogen-activated protein kinase (MAPK) has a role in the generation of neuropathic pain (28).

By blocking $cysLT_1$, montelukast reduced the improvement of nociception by peptide-LTs. Probably, the $cysLT_1$ receptor stimulation interferes in central nociception mechanism in a different way than stimulating $cysLT_2$ receptors from the level of ganglionic roots. In our study, MK administrated as single medication didn't increase significantly the mechano-nociception threshold, but induced a significant anti-nociceptive effect in experimental-induced thermoalgesia (30).

The expression of leukotriene receptors in the rat dorsal root ganglion (15) showed a pathway for the involvement of leukotrienes in neuropathic pain: neurons containing $CysLT_2$ receptors are co-localized with TRPV1 neurons and P2x3 positive neurons. $CysLT_2$ receptor stimulation at the level of dorsal root of ganglion itself did not induce hyperalgesia or spontaneous pain behaviour but the treatment with LTC4 enhanced the painful behavior produced by P2x3 receptor agonists (as beta-methylene adenosine 5´triphosphate) (31). By $cysLT_2$ receptor way peptidoleukotrienes can be involved in the augmentation of the pain behavior. On the other part the activation of both $cysLT_2$ receptors and EP (receptors for prostaglandin E) in the unmyelinated nociceptive fibers from the dorsal root neurons can modulate TRPV1 receptor activity and play a role in neuronal sensitization.

The peripheral nerve injury increased the spinal cord prostaglandins and LTs synthesis. Because both PGE_2 and peptidoleukotrienes potentiate the P2x3 receptor activity in dorsal root ganglion neurons and modulate the TRPV1 receptor activity is possible to be a complex modulator action of the eicosanoids system in the neuropathic pain. The BLT_1 is expressed also in the dorsal root ganglion neurons in mice (32). There is also a direct activation of capsaicin receptors by 5-LOX metabolites as leukotrienes (33).

LTB_4 acts as intracellular mediator at the level of vanilloid type 1 receptor (34). This receptor type is involved in nociception. 5-HPETEs and LTB_4 act as endovanilloids receptors modulator (35).

Some data indicate the possibility of trascellular biosynthesis of cysteinyl-leukotrienes in neural and glial cells (36). The release of spinal glutamate by inflammatory stimuli causes hyperalgesia by NMDA

receptor activation and by sensitization of primary sensory neurons (37). The leukotrienes from CNS could be involved in this process.

The systemic administration of kainate (an agonist of NMDA receptors) in rats induced upregulation of the brain 5-LOX. By this mechanism endogenous glutamate can increase the 5-LOX activity in the CNS (38). NMDA receptors stimulation activates 5-LOX in rats' central neurons (39). By this way the synthesized leukotrienes have a modulating action at TRPV central receptors level. We believe that the eicosanoids system would play a complex modulator role not only in inflammation but also in nociception.

CLINICAL DATA

A strong argues for the involvement of LTs in this process is the increased urinary release of LTs during the pain produced in different diseases. Urinary concentration of cysteinyl-leukotrienes (especially LTE_4) significantly increased in pain, in cases of the patients with sickle cell disease (40). There is an important increase in the urinary clearance of LTE_4, from 82.34pg/mg creatinine in sick but painful patient, up to 162.8 pg/mg during a pain episode. The measurement of the urinary LTE_4 is a non-invasive method. The urinary LTE_4 measurements can be used as a marker for asthma evolution and a susceptibility to the treatment with $cysLT_1$ receptors antagonists, but we consider that the rapid variations of this leukotriene's urinary level can be a biological marker of pain intensity (at least for some types of pain)(and of course of treatment efficacy) (41).

The leukotrienes urine levels are increased in patients with Printzmetal angina. Compared to control healthy group, the urine level of LTE_4 increases from 51.1±21.3pg/mg creatinine (in control group) to 122.7±37pg/mg creatinine in the Printzmetal angina group ($p<0.01$) (42). The level is not increased in patients with chronic stable angina. In experimental model of cardiac ischemia in animals, and also in acute chest pain in patients with the myocardial infarction, the level of urinary LTE_4 excretion increased (43). Because the elevated rates of urinary LTE_4 excretion were measured shortly after the onset of the chest pain, we think that the high level of leukotrienes overlap the highest intensity of the ischemic pain. In experimental ischemia some inhibitors of 5-LOX showed an important reduction in infarct size (44). The ischemia enhances the 5-LOX activity and synthesis of LTs involved in nociception. It is possible that leukotrienes act in this situation by two different ways:

1. involvement in the production of ischemic myocardial damages and the nociceptive production consecutive to synthesis and release of different compounds in the infracted area;
2. direct action at the level of nervous endings.

We suggest that 5-LOX expression in brain is possible to be higher in chronic pain. It is not clear the correlation between the chronic pain and the neurodegeneration, but the 5-LOX expressed in the CNS neurons and nevroglia may be involved also in the neurodegeneration process (45).

THERAPEUTIC PERSPECTIVES

The fact that besides the involvement in pain generation, prostaglandins (the first group of eicosanoids studied) and leukotrienes have an important role in the same process opens many therapeutic ways:

1. The use of $cysLT_1$ antagonists (already therapeutically used as anti-asthma drugs) in the therapy of some types of pain;
2. The association of leukotriene antagonists with COX-1 or COX-2 inhibitors;
3. The production and use in the pain therapy of the 5-LOX selective inhibitors (such as zileuton);
4. The use for the pain treatment of dual inhibitors of 5-LOX and COX-1/COX-2 (such as licofelone) (46);
5. The therapeutic use of cys-LT_1 receptor antagonists is considered to be safe.

A small number of side effects were observed. Montelukast, pranlukast and zafirlukast are well tolerated drugs. The most common side effects are the Churg-Strauss syndrome, gastric discomfort, headache (47). Zileuton (25-100 mg/kg p.o.) and licofelone (10-100 mg/kg p.o.) significantly reversed the both cold allodynia threshold, but the effect of licofelone was stronger. These results indicate that the dual inhibition of these two key enzymes involved in the eicosanoids synthesis could represent a beneficial way in the treatment of hyperalgesia from postoperative pain.

The association between zileuton and zafirlukast inhibited acid acetic-induced writhing in mice. In this case it is interesting that ED50 of zileuton (inhibitor of 5-LOX) was 31,81mg/kg p.o. compared to ED_{50} of zafirlukast (antagonist of receptors) 6,15mg/kg p.o (48). Zileuton was also efficient against experimental carrageenan-induced hyperalgesia. This inhibitor of 5-LOX is also well tolerated and increases the hepatic transaminases level (only in 4.4% from patients receiving 3 months zileuton treatment had elevations in ALT levels) (49). Regarding the the therapeutic use of 5-LOX inhibitors, there are data about the a possible interaction between 5-LOX and nuclear factorNF-kB and on the capability of 5-LOX to influence the process of transcription (50).

Flavocoxid, a dual inhibitor of cyclooxygenase and 5-LOX flavocoxid decreased MDA, TNF and nitrite levels from LPS-stimulated macrophages, inhibits the phospholipase A2 and could be also used as an analgesic drug (51).

Besides the bronchial asthma therapy, the leukotrienes synthesis inhibitors and LTs antagonists will become important therapeutic agents in inflammatory bowel diseases, rheumatoid arthritis, allergic rhinitis and others. There are possibilities to associate in pain therapeutics the anti pain action of resolvins analogs (52) to cys-LT_1 receptors antagonists and to 5-LOX inhibitors.

Finally, the 5-LOX metabolites are involved also in the toxicity of non-steroidal anti-inflammatory drugs (NSAID). NSAID are very used in the treatment of the pain. This group of substances inhibits the COX-1 and the COX-2 and suppresses the prostaglandins and the synthesis but but show no anti-5-LOX activity.

The 5-LOX is a new target for anti-inflammatory drugs and there have perspectives to be also a target for the pain therapy.

REFERENCES

1. Samuelsson B, Funk CD, Hoshiko S, Matsumoto T, Radmark O. Molecular biology and lipoxin formation. In: B. Samuelsson, P.Y.K Wow G. and F.F. Sun (Eds), Advances in Prostaglandins, Thromboxane and Leukotriene Research, Raven Press Ltd. New York, 1989; 19: 1-10.
2. Shi ZZ, Han B, Habib GM, et al. Disruption of gamma-glutamyl leukotrienase results in disruption of leukotriene D4 synthesis in vivo and attenuation of acute inflammatory response. *Mol Cell Biol.* 2001: 21:5389-95.
3. Yamashita M. Peroxisome proliferator-activated receptor alpha-independent effects of peroxisome proliferators on cysteinyl leukotriene production in mast cells. *Eur J Pharmacol* 2007; 556: 172-180.
4. Evans JF. Cysteinyl leukotriene receptors. *Prostagland Lipid Mediat* 2002; 68: 587-597.
5. Heise CE, O'Dowd BF, Figueroa DJ, et al. Characterization of the human cysteinyl leukotriene 2 receptor. *J Biol Chem.* 2000; 275(30): 531-536.
6. Brink C, Dahlén SE, Drazen J, et al. International Union of Pharmacology XLIV. Nomenclature for the oxoeicosanoid receptor. *Pharmacol Rev*. 2004;56:149-157.
7. Mehrabian M, Allayee H, Wong J, et al. Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. *Circ Res*. 2002; 26: 120-126.
8. Rubin P, Mollison KW. Pharmacotherapy of diseases mediated by 5-lipoxygenase pathway eicosanoids *Prostagland Other Lipid Mediat* 2007; 83: 188-197.
9. Riccioni G, Bucciarelli T, Mancini B, et al. Antileukotriene drugs: clinical application, effectiveness and safety. *Curr Med Chem*. 2007; 14: 1966-77.
10. Henderson WR. The role of leukotrienes in inflammation. *Ann. Intern Med* 1994; 121: 684-697.
11. Palmer RMJ, Salmon JA. Inhibition of 5-lipoxygenase: relevance to inflammation; Drugs Affecting Leukotrienes and other Eicosanoid Pathways. In: Samuelsson B, Berti F, Folco GC, Veloso GP Eds. Plenum Press New York, London 1985: 311-330.
12. Trang T, McNaull B, Quirion R, et al. Involvement of spinal lipoxygenase metabolites in hyperalgesia and opioid tolerance. *Eur. J. Pharmacol* 2004; 491: 21-30.
13. Guerrero ATG, Verri WA, Cunha TM, et al. Involvement of LTB4 in zymosan-induced joint nociception in mice:participation of neutrophils and PGE2. *J. Leukocyt Biol* 2008; 83: 112-130.
14. Martin HA, Basbaum AI, Goetzl EJ, et al. Leukotriene B4 decreases the mechanical and thermal thresholds of C-fiber nociceptors in the hairy skin of the rat. *J Neurophysiol*. 1988; 60: 438-445.
15. Tonussi CR, Ferreira SH. Tumour necrosis factor-alpha mediates carrageenin-induced knee-joint incapacitation and also triggers overt nociception in previously inflamed rat knee-joints. *Pain* 1999; 82: 81-87.
16. Cunha JM, Sachs D, Canetti CA, et al. The critical role of leukotriene B4 in antigen-induced mechanical hyperalgesia in immunized rats. *Br.J. Pharmacol* 2003; 139: 1135-45.
17. Masferrer JL, Zweifel BS, Hardy MGD, et al. *J.Pharmacol. Exp.Ther.* 2010; 334: 294-301.
18. Corte FP, Barja-Fidalgo C, Verri WA Jr, et al. Endothelins modulate inflammatory reaction in zymosan-induced arthritis: participation of LTB4, TNF-alpha and CXCL-1. *J.Leukoc. Biol* 2008; 84: 652-660.
19. Carvalho AP, Bezerra MM, Girao VC, et al. Anti-inflammatory and antinociceptive activity of risedronate in experimental pain models in rats and mice. *Clin. Exp. Pharmacol. Physiol*. 2006; 33: 601-606.
20. 20.Woszczek G, Chen LY, Nagineni S, et al. IFN-gamma induces cysteinyl leukotriene receptor 2 expression and enhances the responsiveness of human endothelial cells to cysteinyl leukotrienes. *J Immunol* 2007; 178: 5262-70.
21. Steinke JW, Culp JA. Leukotriene synthesis inhibitors versus antagonists: the pros and cons. *Curr Allergy Asthma Rep* 2007; 7: 126-133.
22. Sherhan CN, Chiang N, VanDyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol* 2009; 8: 349-361.
23. 23.Qu X, Zhang X, Yao J, Song J, Nikolic-Paterson DJ, Li J. Resolvins E1 and D1 inhibit interstitial fibrosis in the obstructed kidney via inhibition of local fibroblast proliferation. *J Pathol* 2012; 228: 506-519.
24. Yavin E, Brand A, Gree P. Docosahexaenoic acid abundance in the brain:a biodevice to combat oxidative stress. *Nutr. Neurosci* 2002; 5: 149-157.
25. Spite M, Norling LV, Summers L, et al. Resolvin D2 is a potent regulator of leukocytes and controls microbials sepsis. *Nature* 2009; 461: 1287-91.
26. 26.Park CK, Xu ZZ, Liu T, et al. Resolvin D2 is a potent endogenous inhibitor for transient receptor potential subtype V1/A1, inflammatory pain, and spinal cord synaptic plasticity in mice: distinct roles of resolvin D1, D2, and E1. *J Neurosci* 2011; 31: 18433-38.
27. Xu ZZ, Zhang L, Liu T, et al. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. *Nat Med*. 2010; 16: 592-597.
28. 28.Noguchi K, Okubo M. Leukotrienes in nociceptive pathway and neuropathic/inflammatory pain. *Biol Pharm Bull.* 2011; 34: 1163-69.
29. Werz O, Steinhilber D. Development of 5-lipoxygenase inhibitors--lessons from cellular enzyme regulation. *Biochem Pharmacol*. 2005; 70: 327-333.
30. Nechifor, Cuciureanu MM, Chelarescu D, et al. Magnesium and other bivalent cations influence upon sodium montelukast effect in experimental-induced thermoalgesia. *Magnes Res* 2008; 21: 38-42.
31. Okubo M, Yamanaka H, Kobayashi K, et al. Expression of leukotriene receptors in the rat dorsal root ganglion and the effects on pain behavior. *Mol. Pain* 2010; 6: 57-65.

32. Andoh T, Kuraishi Y. Expression of BTL1 leukotriene B4 receptors on the dorsal root ganglion neurons in mice. *Mol. Brain Res.* 2005; 137: 260-263.
33. Hwang SW, Cho H, Kwak J, et al. Direct activation of capsaicin receptors by products of lipoxygenases endogenous capsaicin-like substances. *Proc. Nat. Acad. Sci. USA* 2000; 97: 6155-60.
34. Chen H, Dzitoyeva S, Manev H. 5-Lipoxygenase in mouse cerebellar Purkinje cells. *Neuroscience* 2010; 171: 383-389.
35. DePetrocellis L, DiMarzo V. Lipids as regulators of the activity of transient receptor potential type VI (TRPV1) channels. *Life Sci* 2005; 77: 1651-66.
36. Farids SE, Zarini S, Precht T, et al. Transcellular biosynthesis of cysteinyl leukotrienes in rat neuronal and glial cells. *J. Neurochem* 2007; 103: 1310-18.
37. Ferreira SH, Lorenzetti BB. Glutamate spinal retrograde sensitization of primary sensory neurons associated with nociception. *Neuropharmacology* 1994; 33: 1479-85.
38. Manev H, Uz T. Early up-regulation of hypocampal 5-lipoxygenase following systemic administration of kainate to rats. *Restor. Neuro. Neurosci.* 1998; 12: 81-85.
39. Ge QF, Hu X, Ma ZQ, et al. Baicalin attenuates the oxygen-glucose deprivation-induced injury via inhibiting NMDA receptor-mediated 5-lipoxygenase activation in rat cortical neurons. *Pharmacol. Res* 2007; 55: 148-157.
40. Field JJ, Strunk RC, Knight-Perry JE. Urinary cysteinyl leukotriene E4 significantly increase in pain in children and adults with sickle cell disease. *Am.J. Hematol* 2009; 84: 231-233.
41. Rabinovitch N. Urinary leukotriene E4. Immunol. *Allergy Clin. North Am* 2007; 27: 651-664.
42. DeCaterina R, Giannessi D, Lazzenni G, et al. Sulfido-peptide leukotrienes in coronary heart disease-relationship with disease instability and myocardial ischemia. *Eur. J. Clin. Invest* 2010; 40: 258-272.
43. Carry M, Korley V, Willerson JT, Weigelt L, et al. Increased urinary leukotriene excretion in patients with cardiac ischemia.In vivo evidence for 5-Lipoxygenase activation. *Circulation* 1992; 85: 230-236.
44. Mullane K, Hatala MA, Kraemer R, et al. Myocardial salvage induced by REX-590: An inhibitor and antagonist of the leukotrienes. *J. Cardiovascular Pharmacol* 1987: 398-406.
45. Manev H, Uz T, Sugaya K, Qu T. Putative role of neuronal 5-lipoxygenase in an aging brain. *FASEB J.* 2000; 14: 1464-69.
46. Celotti F, Durand T. The metabolic effects of inhibitors of 5-lipoxygenase and of cycloxygenase 1 and 2 are an advancement in the efficacy and safety of anti-inflammatory therapy. *Prostagland.Lipid Mediat* 2003; 71: 147-162.
47. Montuschi P, Sala A, Dahlén SE, et al. Pharmacological modulation of the leukotriene pathway in allergic airway disease. *Drug Discov Today* 2007;12: 404-412
48. Singh VP, Patil CS, Keukarni SK. Effect of licofelone against mechanical hyperalgesic allodynia in the rat model of incisional pain. *Pharmacol. Rep* 2005; 57: 380-384.
49. Watkins PB, Dube LM, Walton-Bowen K, Cameron CM, Kasten LE. Clinical pattern of zileuton-associated liver injury: results of a 12-month study in patients with chronic asthma. *Drug Saf* 2007; 30: 805-815.
50. Lepley RA, Fitzpatrick FA. 5-Lipoxygenase compartmentalization in granulocytic cells is modulated by an internal bipartite nuclear localizing sequence and nuclear factor kappa B complex formation. *Arch Biochem Biophys* 1998; 356: 71-76.
51. Burnett BP, Bitto A, Altavilla D, et al. Flavocoxid inhibits phospholipase A2, peroxidase moieties of the cyclooxygenases (COX), and 5-lipoxygenase, modifies COX-2 gene expression, and acts as an antioxidant. *Mediators Inflamm.* 2011: 385780.
52. Ji RR, Xu ZZ, Strichartz G, et al. Emerging roles of resolvins in the resolution of inflammation and pain. *Trends Neurosci* 2011; 34: 599-609.

LEUKOTRIENELE SI DUREREA – NOI PERSPECTIVE TERAPEUTICE

REZUMAT

Leukotrienele (LTs) sunt un important grup de icosanoizi implicaţi în mecanismul durerii inflamatorii şi neinflamatorii.Implicarea LTs în mecanismul durerii inflamatorii este susţinută de datele ce arată că multi mediatori ai inflamatiei cum sunt TNF, unele interleukine şi alţii stimulează sinteza de LTB_4 şi de peptidoleukotriene.Antagonistii LTB_4 si inhibitorii de 5-lipoxigenază reduc nociceptia inflamatorie de cauză periferică. LTs sunt implicate de asemenea în durerea neuropatică. Activarea receptorilor cys LT_1 din neuronii din cornul dorsal al măduvii poate modula activitate receptorilor TRPV1. Datele noastre experimentale arată că montelukastl (un cysLT_1 antagonist) creşte pragul mecanoalageziei la sobolan. Inhibarea sintezei LTs precum şi blocarea receptorilor BLT_1, BLT_2, cysLT_1 şi cys LT_2 ar putea constitui in viitor o noua cale în terapia durerii.

Cuvinte cheie: leukotriene, durere inflamatorie, durere neuropatică, inhibitori ai 5-LOX, montelukast, risedronat, licofelon

THE INFLUENCE OF INTRACORONARY VERAPAMIL ADMINISTRATION ON LEFT VENTRICULAR FUNCTION IN PATIENTS WITH PREVIOUS MYOCARDIAL INFARCTION TREATED WITH DIRECT STENTING

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ABSTRACT

Objectives: We evaluated the effect of intracoronary verapamil on left ventricular function and prognosis in patients with previous myocardial infarction treated with direct stenting of the artery responsible for infarction by primary percutaneous transluminal coronary angioplasty (PTCA) using ultrasound parameters (EDV-diastolic volume, end systolic volume-ESV; diastolic diameter-EDD, ejection fraction, EF) at admission, 6 weeks and 1 year after.

Background: Clinical trials to date have documented verapamil's potential to increase coronary blood flow after primary PTCA.

Methods: We conducted a retrospective analysis of 56 consecutive patients with a first anterior AMI who underwent direct stenting of the lesion responsible for infarction; they were randomized into verapamil group (n = 28) or control group (n = 28). In the first group, 500ug verapamil were injected (2 ml from a dilution of 5 mg in 20ml of saline)) through guide catheter in the coronary artery (LAD) 1 minute after direct stenting of the lesion responsible for infarction, whereas in the control group were given 2 ml of saline. To evaluate the effect of intracoronary verapamil administration on left ventricular function, the following ultrasound parameters were assessed (EDD, EDV, ESV, EF) on admission, at 6 weeks and 1 year.

Results: The two groups were homogeneous in terms of age, gender, systolic blood pressure, diastolic blood pressure, heart rate and cardiovascular risk factors. No statistically significant differences were noted on EDD, EDV, ESV, EF at the echocardiography performed on admission.

6 weeks after the first echocardiographic evaluation the patients from control-group presented unfavourable evolution after stenting with statistically significant increases of EDD ($p = 0.001$), EDV ($p = 0.002$), ESV ($p = 0.004$) and a statistically significant decrease for EF ($p = 0.0015$), while in the verapamil-group they had a positive evolution with statistically insignificant slow increase of EDD ($p = 0.083$), EDV ($p = 0.9$), and a statistically insignificant decrease of ESV ($p = 0.52$) with a highly statistically significant increase of EF ($p <0.001$).

1 year after the second echocardiography, patients in the control group had an negative outcome after stenting with statistically significant increases for EDD ($p <0.001$), EDV ($p = 0.002$), ESV ($p = 0.003$) and a statistically insignificant decrease of EF ($p = 0.062$), while in the verapamil-group had a favourable evolution with a constant growth statistically insignificant of EDD ($p = 0.085$), EDV ($p = 0.878$), a statistically insignificant decrease of ESV ($p = 0.379$) and a highly statistically significant increase of EF ($p <0.001$).

Patients from control group, at 1 year compared to 6 weeks had a highly statistically significant ($p <0.001$) increase of EDD and a statistically significant increase of EDV ($p = 0.007$) and ESV ($p = 0.022$). EF was insignificantly increased ($p = 0.506$) at 1 year compared to 6 weeks.

In patients from verapamil-group at 1 year compared to 6 weeks no statistically significant differences regarding EDD ($p = 0.151$), EDV ($p = 0.928$), ESV ($p = 0.251$) were found. Regarding EF, there was a statistically significant increase ($p = 0.003$) at 1 year compared to 6 weeks.

Conclusions: Intracoronary administration of verapamil after primary PTCA can improve left ventricular function most likely by reducing microvascular dysfunction and therefore, by increasing myocardial blood flow in patients with AMI, leading to better outcomes compared to patients treated only with PTCA. Thus, increasing myocardial blood flow in patients with AMI, intracoronary verapamil administration prevents left ventricular negative remodelling, which could explain the positive long-term outcomes in patients with AMI who received intracoronary verapamil.

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INTRODUCTION

Heart attacks are fatal in about one third of the cases. Because acute myocardial infarction can occur in the most productive years of activity of an individual, significant negative consequences are possible both in terms of psychosocial and economical outcomes (1).

Today there are many options for reperfusion such as pharmacological or mechanical, which may be different in terms of efficacy. The success of reperfusion therapy in acute myocardial infarction depends on the ability of obtaining complete and rapid restoration of antegrade blood flow in the infarction artery. In patients with acute myocardial infarction the favourite approach is primary PTCA (2). However, epicardial reperfusion after this procedure is not able to stop immediately the myocardium impairment. The strategies are geared toward improving myocardial perfusion on a tissue level because that is affected in approximately 70% of the cases even after angioplasty, and therefore afflicts prognosis.

Out of all types of acute myocardial infarction, the anterior AMI, involving the anterior descending artery and a wider territory exposed to infarction risk, has the greatest impact on prognosis and left ventricular function.

MATERIAL AND METHODS

In this study were included 56 consecutive patients with acute anterior myocardial infarction treated by direct stenting of the infarction lesion, hospitalized in the Institute of Cardiovascular Diseases Timisoara between February 2012 and October 2013. Included patients were diagnosed with acute anterior myocardial infarction without known history of other heart attacks, with thrombotic occlusion or stenosis with superimposed thrombus in the proximal and middle third of the anterior descending artery, undergoing percutaneous transluminal coronary angioplasty with direct stenting of the lesion responsible for infarction in the first 12 hours of the symptomatology onset.

Patients were randomized into verapamil group (n = 28) and control group (n = 28). In the first group, 500ug verapamil (2 ml out of 5mg dilution in 20ml of saline) were injected into the infarction artery through guide catheter 1 minute after direct stenting. Choosing a dosage of 500ug verapamil was based on findings from other studies with intracoronary[3] verapamil administration. In the control group 2 ml of saline were administered through guide catheter 1 minute after direct stenting of the infarction lesion.

Medication management protocol: prior to PTCA, all patients were treated with acetylsalicylic acid 250mg, clopidogrel 600 mg and after PTCA, 100 mg / day acetylsalicylic acid and 150 mg / day clopidogrel for one month, then 75mg / day up to one year; all patients received unfractionated heparin between 3 and 5 days after PTCA. All patients were echocardiographic assessed in the first 24 hours from admission and key parameters were evaluated (end-diastolic diameter - EDD, end-diastolic volume - EDV, end systolic volume - ESV, ejection fraction - EF).

The study lasted over 1 year. Patients were evaluated at 6 weeks, 3 months, 6 months, 9 months and 1 year. At 6 weeks and 1 year they repeated the echocardiography. To determine the effect of intracoronary verapamil administration on left ventricular function the key ultrasound parameters were assessed at admission, at 6 weeks and 1 year.

The angiography was performed by femoral or radial approach in the day of admission. After diagnostic coronary angiography by which the lesion located in the in the proximal or medial 1/3 of the anterior descending artery responsible for infarction was identified (hemodynamically significant stenosis with thrombus superimposed or thrombotic occlusion) the next step was direct stenting of the lesion. In verapamil-group, 500ug verapamil (2 ml out of 5mg dilution in 20ml of saline) were injected into the infarction artery through guide catheter 1 minute after direct stenting, whether or not "no reflow" phenomenon was present. There was no significant change in any variable (SBP, DBP and HR) before and after administration of intracoronary verapamil. No significant hemodynamic abnormality, shock or malignant ventricular arrhythmia was observed during or after administration of intracoronary verapamil.

ETHICAL CONSIDERATIONS

All patients were instructed on entrance into the study, and each patient signed an informed consent. The study was based on international standards of medical ethics regarding patient's confidentiality, established by the Declaration of Helsinki.

STATISTICAL ANALYSIS

For numerical variables 2 by 2 for the 3 moments of evaluation, we used t test, and for ordinal variables we utilized signed-rank Wilcoxon test. The significance threshold was 0.05 (5%), corresponding to a 95% confidence level. Depending on the p value, the results were interpreted as: $p \geq 0.05$ - insignificant differences, $p < 0.05$ - significant differences, $p < 0.01$ – very significant differences; $p < 0.001$ - highly significant differences.

RESULTS

To assess the outcomes for the patients in the control group, ultrasound parameters (EDD, ESV, EDV and EF) were compared on admission, at 6 weeks and 1 year. Also, ultrasound parameters were analysed at 6 weeks compared to 1 year. The results are in Table I.

Table I. Echocardiography parameters in patients in control group at admission (start), 6 weeks (6W) and 1 year.

Match		Mean	N	Std. deviation	Mean standard deviation	p
1	Start-EDD cm	4.78	28	0.551	0.147	0.001
	6 W EDD cm	5.02	28	0.531	0.142	
2	6 W EDD cm	5.02	28	0.531	0.142	<0.001
	1year EDD cm	5.32	28	0.430	0.119	
3	Start-EDD cm	4.78	28	0.551	0.147	<0.001
	1year EDD cm	5.32	28	0.430	0.119	
4	Start-EDV ml	128.86	28	33.222	8.879	0.002
	6W EDV ml	151.07	28	40.912	10.934	
5	6W EDV ml	151.07	28	40.912	10.934	0.007
	1year EDV ml	166.15	28	47.353	13.133	
6	Start- EDV ml	128.86	28	33.222	8.879	0.002
	1year EDV ml	166.15	28	47.353	13.133	
7	Start-ESV ml	77.21	28	22.807	6.096	0.004
	6W ESV ml	93.21	28	29.652	7.925	
8	6W ESV ml	93.21	28	29.652	7.925	0.022
	1year ESV ml	102.46	28	37.854	10.499	
9	Start-ESV ml	77.21	28	22.807	6.096	0.003
	1year ESV ml	102.46	28	37.854	10.499	
10	EF (start)	40.14	28	6.347	1.696	0.015
	EF (6W)	37.64	28	6.134	1.640	
11	EF (6W)	37.64	28	6.134	1.640	0.506
	1year EF	39.00	28	5.745	1.593	
12	EF (start)	40.14	28	6.347	1.696	0.062
	1year EF	39.00	28	5.745	1.593	

Table I shows significant increases over time for EDD (start EDD-6W EDD, p = 0.001; start EDD - 1 year EDD, p <0.001), EDV (start EDV – 6W EDV, p = 0.002; start EDV - 1 year EDV, p = 0.002), ESV (start ESV – 6W ESV, p = 0.004; start ESV - 1 year ESV, p = 0.003). EF at 6 weeks was significantly decreased (p = 0.015) compared to 6 weeks; EF at 1 year compared to admission wasn't statistically significant decreased. At 1 year compared to 6 weeks, there was a highly statistically significant increase of EDD (p <0.001), EDV (p = 0.007) and ESV (p = 0.022). EF didn't have a statistically significant increase (p = 0.506) at 1 year compared to 6 weeks.

These data prove a slowly bad evolution in patients from control group after stenting, heading towards ventricular dilatation and systolic dysfunction, with significant changes in cardiac function with repercussions on the patient's symptoms.

In order to track the evolution of patients in verapamil group, ultrasound parameters (EDD, ESV, EDV and EF) were compared on admission, at 6 weeks and 1 year. The ultrasound parameters were also interpreted at 6 weeks compared to 1 year. The findings are in Table II.

Table II. Echocardiography parameters in patients in verapamil group at admission (start), 6 weeks (6W) and 1 year.

Match		Mean	N	Standard deviation	Mean standard deviation	p
1	Start-EDD cm	4.65	28	0.392	0.105	0.083
	6 W EDD cm	4.78	28	0.314	0.084	
2	6 W EDD cm	4.78	28	0.314	0.084	0.151
	1year EDD cm	4.73	28	0.367	0.098	
3	Start-EDD cm	4.65	28	0.392	0.105	0.085
	1 year EDD cm	4.73	28	0.367	0.098	
4	Start-EDV ml	116.93	28	25.605	6.843	0.900
	6W EDV ml	117.29	28	20.514	5.483	
5	6W EDV ml	117.29	28	20.514	5.483	0.928
	1yearEDV ml	117.50	28	14.643	3.914	
6	Start-EDV ml	116.93	28	25.605	6.843	0.878
	1year EDV ml	117.50	28	14.643	3.914	
7	Start-ESV ml	67.86	28	14.411	3.851	0.520
	6W ESV ml	64.64	28	11.567	3.091	
8	6W ESV ml	64.64	28	11.567	3.091	0.251
	1year ESV ml	63.93	28	7.869	2.103	
9	Start-ESV ml	67.86	28	14.411	3.851	0.379
	1year ESV ml	63.93	28	7.869	2.103	
10	EF (start)	40.36	28	3.650	0.976	<0.001
	EF (6W)	44.64	28	4.217	1.127	
11	EF (6W)	44.64	28	4.217	1.127	0.003
	1year EF	46.43	28	4.620	1.235	
12	EF (start)	40.36	28	3.650	0.976	<0.001
	1year EF	46.43	28	4.620	1.235	

Table II shows that were obtained statistically insignificantly increases in time for EDD (start EDD-6W EDD, p = 0.083; start EDD - 1 year EDD, p = 0.085) and EDV (start EDV – 6W EDV, p = 0.9; start EDV - 1 year EDV, p = 0.878), a statistically insignificant decrease of ESV (start ESV – 6W ESV, p = 0.52; start ESV - 1 year ESV, p = 0.379), and an increased EF highly statistically significant at 6 weeks and 1 year (p <0.001). At 1 year compared to 6 weeks there weren't statistically significant differences for EDD (p = 0.151), EDV (p = 0.928), ESV (p = 0.251). EF had a statistically significant increase (p = 0.003) at 1 year compared to 6 weeks.

These results prove that patients in verapamil group had a slowly favourable progression after stenting, without significant changes in left ventricular end-diastolic

volume, with significant decreases in left ventricular end systolic volume and consequently a significant increase in ejection fraction compared at 6 weeks with admission. These results, meaning the absence of significant changes in left ventricular end-diastolic volume, significantly decreased end systolic volume, significantly increased left ventricular ejection, were maintained at 1 year compared to admission. There weren't any significant differences in patient outcomes in verapamil group (EDV, ESV and EF) at 1 year compared to 6 weeks. Most likely, a possible explanation could be intracoronary verapamil administration during angioplasty procedure, medication with important role on left ventricular remodelling, remodelling that occurs in the first month after myocardial infarction.

DISCUSSIONS

Percutaneous coronary angioplasty is the main reperfusion strategy for acute coronary syndromes (2). Initially, it was thought that its benefit is due to timely restoration of blood flow to the ischemic myocardium above. Despite current evidence in favour of PTCA, the no-reflow phenomenon can happen and is associated with a poor prognosis both in hospital and long term (4).

No reflow concept means hypoperfusion of the myocardial tissue, due to coronary microcirculation dysfunction in the presence of opened epicardial coronary arteries. No reflow phenomenon is an independent predictor of both short- and long-term adverse cardiac events and mortality following PTCA (5). The existence of no reflow phenomenon was initially a debate theme; however, multiple experimental data and clinical studies have clearly demonstrated that it occurs after reperfusion with a variable prevalence ranging from 5% to 50%, in accordance with the methods used to evaluate the phenomenon and the analysed population (6,7).

Patients who develop no-reflow have a higher prevalence of: 1) early post-infarction complications (arrhythmias, pericardial effusion, cardiac tamponade, early congestive heart failure); 2) negative remodelling of the left ventricle; 3) rehospitalisation for acute decompensated heart failure; and 4) mortality.

From a pathophysiological point of view it can be divided into two categories: reperfusion no-reflow and intervention no-reflow. Reperfusion no-reflow may occur after opening an epicardial coronary artery that closed suddenly, for example during thrombolysis for STEMI, but in fact, the most common cause is PTCA performed in a STEMI. Intervention no-reflow is defined as an impairment or lack of perfusion of myocardial tissue after ballooning or stenting, provided that no significant residual stenosis, dissection, thrombus or spasm exists. It can happen during PTCA on native vessels or, more commonly, during PTCA on vein grafts. No-reflow phenomenon is multifactorial with many pathological changes that eventually lead to damaged microcirculation and impaired myocardial perfusion (8,9). Reperfusion no-reflow is mainly associated with ischemia reperfusion injury, inflammatory activity and embolization of thrombotic and atherosclerotic material. Interventional no-reflow is triggered by local tissue damage and embolization of atherosclerotic material into microcirculation during PTCA. The main factors contributing to the no-reflow phenomenon are: cellular oedema, ischemia reperfusion injury, microvascular spasm, obstruction of capillaries with neutrophils, microemboli, activation of the extrinsic pathway of coagulation.

Although the negative effects are well known, no specific technique is currently recommended in guidelines to prevent distal embolization during PTCA. Direct stenting and avoiding thrombus fragmentation by ballooning and fixing thrombotic material in the stent wall, was suggested as a possible technique to reduce distal embolization. One randomized study (10) showed improvement of reperfusion in patients selected for direct stenting compared with standard PTCA.

A promising technical approach to prevent no-reflow during mechanical reperfusion is the use of thrombectomy devices and distal filters. Thrombectomy suction catheter is recommended in any STEMI with high thrombotic load as it improves clinical outcomes compared with PTCA alone and reduces especially the reperfusion no-reflow by 52% (11,12). Mechanical thrombectomy (with devices for fragmentation and suction of the thrombus) had controversial results and is not generally recommended. Distal protection devices shown good results in PTCA for saphenous vein grafts, improving clinical and angiographic results, but failed to enhance clinical outcomes during PTCA for AMI on native (11,13) vessels.

Current therapy of no-reflow phenomenon includes vasodilators, antithrombotic drugs medication and trombolitics (14-16). Adenosine is regarded as an effective drug for reducing the incidence of no-reflow phenomenon; however, it may increase the risk of heart block, hypotension, and other side effects. Moreover, the half-life is relatively short, and so the agent should be repeatedly administered (17). In addition, sodium nitroprusside improves blood flow and prevents coronary no-reflow, but the vasodilator effect can cause a dose-dependent hypotension, which limits its clinical usefulness (18,19). In contrast to these drugs, verapamil not only prevents the occurrence of no-reflow, but also improves clinical outcomes on short term. However, further clinical studies on a large scale are needed to assess the broad utility of the agent and long-term prognosis, but verapamil could be a more promising adjunctive therapy than other agents currently available.

Vasodilator drugs can be given before (either routinely or in selected patients) or after PTCA procedure. In practice, these drugs are considered only in patients with slow coronary blood flow.

In our study, intracoronary administration of verapamil resulted in a reduction of the infarction area, a

more favourable left ventricular remodelling, meaning the absence of left ventricular dilatation at 6 weeks and 1 year compared to admission, and increased ejection fraction of left ventricle at 6 weeks and 1 year compared to admission. Intracoronary verapamil administration exerts its effect by dilating mostly resistance arterioles and reducing calcium influx in cells of the myocardium with ischemia. Our data indicate that the coronary blood flow improvement has been associated with a decrease in myocardial infarct size, indicating attenuation of postischemic microvascular dysfunction. This enhancement was noticed in the penumbra area especially where myocardial perfusion is partially preserved. Thus, intracoronary verapamil administration can alleviate microvascular dysfunction, especially in the decreased blood flow area in patients with AMI treated with PTCA, and this attenuation may be associated with an increase in coronary blood flow.

CONCLUSIONS

Patients in the verapamil group had better outcomes than patients in the control group, accordingly to results at 6 weeks compared to admission, which remained favourable at 1 year compared to admission in patients from verapamil group. The administration of intracoronary verapamil resulted in a more favourable left ventricular remodelling, meaning the absence of left ventricular dilatation at 6 weeks and 1 year compared to admission, and increased left ventricular ejection fraction at 6 weeks and 1 year compared to admission.

No significant differences in patient outcomes were noted in verapamil group (EDV, ESV and EF) at 1 year compared to 6 weeks. This could be explained by administration of intracoronary verapamil during primary PTCA procedure, a medication with an important role in left ventricle remodelling, remodelling that occurs in the first month after myocardial infarction.

CLINICAL IMPLICATIONS

Success or failure of a coronary intervention should ideally be assessed by myocardial perfusion recovery. However, the end-point of several studies was angiographic patency of the artery responsible for infarction. Our results clearly indicate that a patent artery does not necessarily guarantee the permeability of coronary microcirculation in patients with AMI. The administration of intracoronary verapamil can partially recover microcirculation integrity and enhance coronary blood flow, leading to a better recovery of the myocardial function. Therefore, epicardial coronary artery recanalization should not be the only goal. Pharmacological adjuvant treatment (intracoronary verapamil) may protect coronary microcirculation against ischemic damage, realising an optimal myocardial saving for patients with AMI.

REFERENCES

1. Mann & Zipes & Libby & Bonow: Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine, 10th Edition. Elsevier Saunders 2014.
2. Hendler A, Aronovich A, Kaluski E, et al. Optimization of myocardial perfusion after primary coronary angioplasty following an acute myocardial infarction. Beyond TIMI 3 flow. *J Invasive Cardiol.* 2006; 18(1): 32-6.
3. Su Q1, Li L, Liu Y. Short-term effect of verapamil on coronary no-reflow associated with percutaneous coronary intervention in patients with acute coronary syndrome: a systematic review and meta-analysis of randomized controlled trials. *Clin Cardiol.* 2013; 36(8): E11-6.
4. Dong-bao L, Qi H, Zhi L, et al. Predictors and long-term prognosis of angiographic slow/no-reflow phenomenon during emergency percutaneous coronary intervention for ST-elevated acute myocardial infarction. *Clin Cardiol.* 2010; 33: E7-E12.
5. Chen Y, Yang Y. No-reflow phenomenon during percutaneous coronary intervention. *Adv Cardiovasc Dis.* 2005; 26:5.
6. Rezkalla SH, Kloner RA. Coronary no-reflow phenomenon: from the experimental laboratory to the cardiac catheterization laboratory. *Catheter Cardiovasc Interv* 2008; 72: 950-7.
7. Eeckhout E, Kern MJ. The coronary no-reflow phenomenon: a review of mechanisms and therapies. *Eur Heart J*. 2001; 22: 729-39.
8. Rezkalla SH, Kloner RA. No-reflow phenomenon. *Circulation* 2002; 105(5): 656-62.
9. Galiuto L. Optimal therapeutic strategies in the setting of postinfarct no reflow: the need for a pathogenetic classification. *Heart* 2004; 90(2): 123-125.
10. Loubeyre C, Morice MC, Lefèvre T, Piéchaud JF, Louvard Y, Dumas P. A randomized comparison of direct stenting with conventional stent implantation in selected patients with acute myocardial infarction. *J Am Coll Cardiol.* 2002; 39: 15-21.
11. Sobieraj DM, White CM, Kluger J, Tongbram V, Colby J, Chen WT, Makanji SS, Lee S, Ashaye A, Coleman CI. Systematic review: comparative effectiveness of adjunctive devices in patients with ST-segment elevation myocardial infarction undergoing percutaneous coronary intervention of native vessels. *BMC Cardiovasc. Disord*. 2011; 11: 74.
12. Vlaar PJ, Svilaas T, van der Horst IC, Diercks GF, Fokkema ML, de Smet BJ, et al. Cardiac death and reinfarction after 1 year in the Thrombus Aspiration during Percutaneous coronary intervention in Acute myocardial infarction Study (TAPAS): a 1-year follow-up study. *Lancet* 2008; 371: 1915-20.
13. Stone GW, Webb J, Cox DA, Brodie BR, Qureshi M, Kalynych A, et al. Distal microcirculatory protection during percutaneous coronary intervention in acute ST-segment elevation myocardial infarction: a randomized controlled trial. *JAMA* 2005; 293(9): 1063-72.
14. Micari A, Belcik TA, Balcells EA, et al. Improvement in microvascular reflow and reduction of infarct size with adenosine in patients undergoing primary coronary stenting. *Am J Cardiol.* 2005; 96: 1410-15.
15. de Lemos JA, Antman EM, Gibson CM, et al. Abciximab improves both epicardial flow and myocardial reperfusion in ST-elevation myocardial infarction. Observations from the TIMI 14 trial. *Circulation.* 2000; 101: 239-243.
16. Forman MB, Jackson EK. Importance of tissue perfusion in ST segment elevation myocardial infarction patients undergoing

reperfusion strategies: role of adenosine. *Clin Cardiol.* 2007; 30: 583-585.
17. Marzilli M, Orsini E, Marraccini P, et al. Beneficial effects of intracoronary adenosine as adjunct to primary angioplasty in acute myocardial infarction. *Circulation.* 2000; 101: 2154-59.
18. Barcin C, Denktas AE, Lennon RJ, et al. Comparison of combination therapy of adenosine and nitroprusside with adenosine alone in the treatment of angiographic no-reflow phenomenon. *Catheter Cardiovasc Interv.* 2004; 61: 484-491.
19. Hillegass WB, Dean NA, Liao L, et al. Treatment of no-reflow and impaired flow with the nitric oxide donor nitroprusside following percutaneous coronary interventions: initial human clinical experience. *J Am Coll Cardiol.* 2001; 37: 1335-43.

INFLUENTA VERAPAMILULUI ADMINISTRAT INTRACORONARIAN ASUPRA FUNCTIEI VENTRICULULUI STANG LA PACIENTII CU INFARCT MIOCARDIC ACUT ANTERIOR TRATATI PRIN STENTARE DIRECTA

REZUMAT

Obiective: am evaluat efectul administarii de verapamil intracoronarian asupra prognosticului si functiei ventriculului stang la pacientii cu infarct miocardic acut anterior la care s-a efectuat stentarea directa a leziunii responsabile de infarct ca tehnica de angioplastie coronariana percutana transluminala(PTCA) primara, prin compararea parametrilor ecografici(volum telediastolic-VTD; volul telesistolic-VTS; diametru telediastolic-DTD; fractia de ejectie-FE) la internare, 6 saptamani si 1 an.
Context: Studii clinice efectuate pana in prezent au documentat potențialul verapamilului de a crește fluxul sanguin coronarian după PTCA primară.
Metode: In perioada februarie 2012 - octombrie 2013, 56 de pacienti consecutivi cu un prim IMA anterior la care s-a efectuat stentarea directa a leziunii responsabile de infarct au fost repartizati aleator la grupul verapamil (n = 28), sau la grupul control (n = 28). In grupul verapamil, a fost injectat verapamil (dilutie 5mg in 20ml de ser fiziologic) 500ug (2ml din dilutie) pe categhid în artera afectată de infarct (LAD) la 1 minut după realizarea stentarii directe a leziunii responsabile de infarct, pe cand in grupul control s-au administrat 2 ml de ser fiziologic. Pentru a evalua efectul verapamilului administrat intracoronarian asupra functiei ventriculului stang s-au urmarit parametrii ecografici(DTD, VTD, VTS, FE) la internare, la 6 saptamani si la 1 an.
Rezultate: Cele doua grupuri au fost omogene din punct de vedere al varstei, sexului, tensiunii arteriale sistolice, tensiunii arteriale diastolice, frecventei cardiace si al factorilor de risc cardiovasculari. Ecografia cardiaca efectuata la internare nu a evidentiat diferente semnificative statistic in privinta DTD, VTD, VTS si FE.
La 6 saptamani dupa prima reevaluare ecocardiografica s-a constatat o evolutie nefavorabila dupa stentare a pacientilor din grupul control cu cresteri semnificative statistic pentru DTD (p=0.001), VTD (p=0.002), VTS (p=0.004) si o scadere semnificativa statistic pentru FE (p=0.0015), pe cand cei din grupul verapamil au avut o evolutie lent favorabila cu cresteri nesemnificative statistic ale DTD (p=0.083), VTD (p=0.9), o scadere nesemnificativa statistic a VTS (p=0.52) si o crestere extrem de semnificativa statistic a FE (p<0.001).
La 1 an dupa a doua reevaluare ecocardiografica s-a constatat o evolutie nefavorabila dupa stentare a pacientilor din grupul control cu cresteri semnificative statistic pentru DTD (p<0.001), VTD (p=0.002), VTS (p=0.003) si o scadere nesemnificativa statistic pentru FE (p=0.062), pe cand cei din grupul verapamil au avut o evolutie constant favorabila cu cresteri nesemnificative statistic ale DTD (p=0.085), VTD (p=0.878), o scadere nesemnificativa statistic a VTS (p=0.379) si o crestere extrem de semnificativa statistic a FE (p<0.001).
In ceea ce priveste rezultatele la 1 an fata de 6 saptamani la pacientii din grupul control se constata o crestere extrem de semnificativa statistic a DTD (p<0.001), precum si o crestere semnificativa statistic a VTD (p=0.007) si VTS (p=0.022). In ceea ce priveste FE se constata o crestere nesemnificativa statistic (p=0.506) la 1 an fata de 6 saptamani.
In ceea ce priveste rezultatele la 1 an fata de 6 saptamani la pacientii din grupul verapamil nu se constata diferente semnificative statistic in privinta DTD (p=0.151), VTD (p=0.928), VTS (p=0.251). In ceea ce priveste FE se constata o crestere semnificativa statistic (p=0.003) la 1 an fata de 6 saptamani.
Concluzii: Administrarea intracoronariana de verapamil după PTCA primară poate imbunatati functia ventriculului stang probabil prin atenuarea disfunctiei microvasculare și, prin urmare, prin cresterea fluxului de sange miocardic la pacientii cu IMA, ceea ce duce la rezultate mai bune decât in cazul pacientilor tratati numai cu PTCA. De asemenea, prin cresterea fluxului de sange intramiocardic la pacientii cu IMA, administrarea intracoronariana de verapamil ajuta la remodelajul ventriculului stang, ceea ce poate explica evolutia mai favorabila pe termen lung a pacientiilor cu IMA la care s-a administrat verapamil intracoronarian.

ANTIBIOTIC CONSUMPTION DATA IN AN INTENSIVE CARE UNIT

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ABSTRACT

Patients admitted to intensive care units (ICUs) are at a greater risk of hospital acquired infection (H.A.I.) than other hospitalized patients and are more likely to die of that infection as a result of a multitude of compounding factors. Patients admitted to ICU's are usually critically ill and require frequent monitoring; rates of infections vary widely depending on the type of ICU.

The study took place in the ICU department of the Timisoara Emergency Clinical County Hospital (TECCH), Romania. Data were recorded for 18 months, between 01.01.2012 and 30.06.2013, for all ICU admitted patients who received antibiotic treatment. For each patient age, gender, admission diagnosis, origin, evolution, type and quantity of antimicrobial agents used (in the ICU) as well as the number of treatment days for each type of antibiotic were recorded.

Data on the number of patients in the ICU, as well as the number of antimicrobials and administration routes were recorded for 1186 patients, with a mean age of 61.78 years (age range between 4 months and 91 years). Based upon the WHO classification system we obtained a total of 911.24 DDD. Out of 1186 patients treated with antimicrobials who were studied during the 18 months period in the TECCH-ICU, 504 received prophylactic treatment, 462 were treated for the underlying pathology and 220 for hospital acquired infection.

The most frequently used classes of chemotherapeutic drugs were third generation cephalosporins, followed by the second generation, represented by cefuroxime alone, the fifth generation on the third position represented by ceftaroline and, finally, the fourth generation represented by cefpirome. These were used for prophylactic purposes and for treating underlying infectious pathology, while carbapenems were especially involved in treating HAI.

Key words: antimicrobials, intensive care units (ICUs), hospital acquired infection (HAI)

INTRODUCTION

In the last decade, it has become possible to treat many patients who could have been lost early at intensive care units (ICU) in the past, thanks to the progress in medical developments and improvement in patient care services (1).

Patients admitted to intensive care units (ICUs) are at a greater risk of hospital acquired infection (H.A.I.) than other hospitalized patients and are more likely to die of that infection as a result of a multitude of compounding factors. Patients admitted to ICU's are usually critically ill and require frequent monitoring; rates of infections vary widely depending on the type of ICU (2-7).

Antibiotics have contributed greatly to improvements in health but the irrational consumption of these preparations lead to more cases of bacterial germs resistant to usual treatments (5-10).

Data from the Surveillance System Intensive Care Antimicrobial Resistance Epidemiology (ICARE) and the Antimicrobial Use and Resistance (AUR) component of the US National Nosocomial Infections Surveillance (NNIS) System show that for most antimicrobial agents, the rate of use was highest in the intensive care area and that consumption ran parallel to the pattern seen for resistance (8).

The aim of this study was to observe the antibiotic consumption in an ICU and to corroborate the above information with a comparative analysis of patient status upon hospital discharge.

MATERIALS AND METHODS

The study took place in the ICU department of the Timisoara Emergency Clinical County Hospital (TECCH), Romania. Data were recorded for 18 months, between 01.01.2012 and 30.06.2013, for all ICU admitted patients who received antibiotic treatment.

For each patient age, gender, admission diagnosis, origin, evolution, type and quantity of antimicrobial agents used (in the ICU) as well as the number of treatment days for each type of antibiotic were recorded.

The amount of antimicrobial drugs was standardized by conversion to defined daily doses (DDD) according to the Anatomical Therapeutic Chemical Classification system (ATC) as defined by the World Health Organization (WHO: www.whooc.no). A DDD is defined as the assumed average maintenance dose per day for a

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drug used for its main indication in adults, but does not necessarily reflect the recommended or prescribed daily dose (11,12).

Statistics

The pooled number of grams for each antibiotic applied by the ICU was divided by the number of grams per DDD for a specified antibiotic, divided by the number of patient-days of this ICU and multiplied by 1,000 to derive the number of DDD per 1,000 patient-days or the antimicrobial use density (AD). Consumption was calculated separately for prophylaxis, treatment of the underlying pathology and hospital acquired infection (HAI).

RESULTS

Data on the number of patients in the ICU, as well as the number of antimicrobials and administration routes were recorded for 1186 patients, with a mean age of 61.78 years (age range between 4 months and 91 years). Based upon the WHO classification system we obtained a total of 911.24 DDD.

Out of 1186 patients treated with antimicrobials who were studied during the 18 months period in the TECCH-ICU, 504 received prophylactic treatment, 462 were treated for the underlying pathology and 220 for hospital acquired infection.

Population analysis revealed the following gender distribution: 58.90% male and 41.10% female.

The mean age of patients receiving prophylactic treatment was 60.11 years and, as expected, more than half of the antibiotics used belonged to the class of cephalosporins (56.48%), followed by fluoroquinolones (9.68%) and penicillins (9.05%).

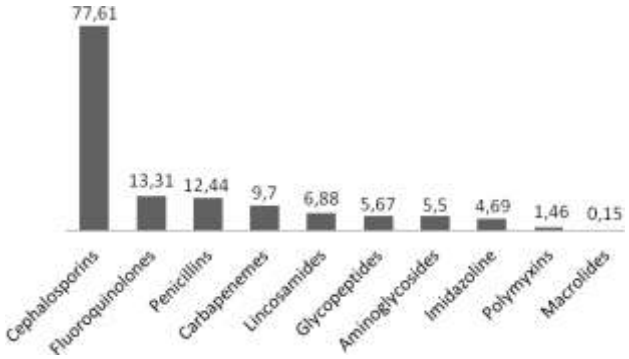

Fig.1. The antimicrobial groups prescribed for prophylaxis purposes in the ICU during the studied period.

The consumption for each antibiotic class was defined as the proportion between the number of DDDs and the number of patient-days.

For the treatment of the underlying pathology, the mean age of the monitored patients was 63.05 years and here the situation regarding the types of antibiotics was similar, cephalosporins being also the most frequently used, with 96.66 DDD per patient-days, but this time closely followed by carbapenems with 88.92 DDD per patient-days as shown in the Figure 2.

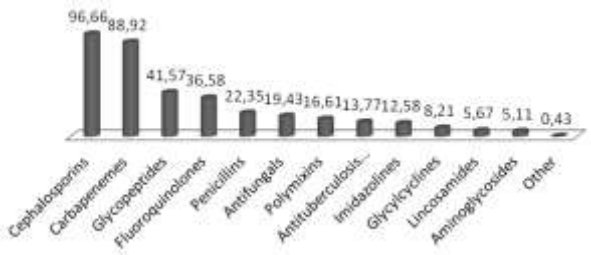

Fig.2. The DDD per patient-days for the classes of antimicrobials used for the treatment of the underlying pathology in the ICU. Legend: "Other" antimicrobials include: sulfonamides, oxazolidindiones, aminophenols.

For the treatment of hospital acquired infections (HAI), the main classes of antimicrobials used were carbapenemes, cephalosporins and glycopeptides. After comparing with the two above mentioned circumstances, we observed that the intensively used class of cephalosporins was exceeded by carbapenems (Figure 3).

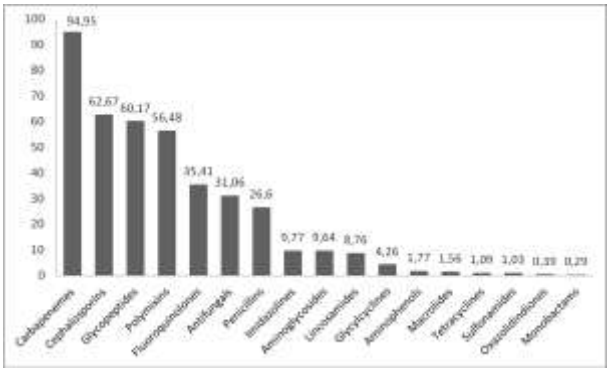

Fig.3. Incidence of antimicrobial classes used in the treatment of HAI expressed as DDDs per patient-days.

The following carbepenems were used in the studied ICU: Meropenem, Imipenem and Ertapenem.

The next figure (Figure 4) presents the antibiotic consumption for HAI, expressed as active substances in order to highlight the most used molecules during the given period. As shown below, Colistin, Ceftriaxone and Meropenem were extensively used.

We also aimed to corroborate the above information with a comparative analysis of patient status upon hospital discharge. Of the patients who received prophylactic treatment, 66.30% left the ICU with an improved status but in 29.80% the evolution was lethal, whereas over 50% mortality was recorded in the groups treated for underlying pathology and for HAI, respectively

i.e. 52.6% deaths in the group treated for underlying pathology and, as expected, a higher percent of deaths in the HAI group (59.10%).

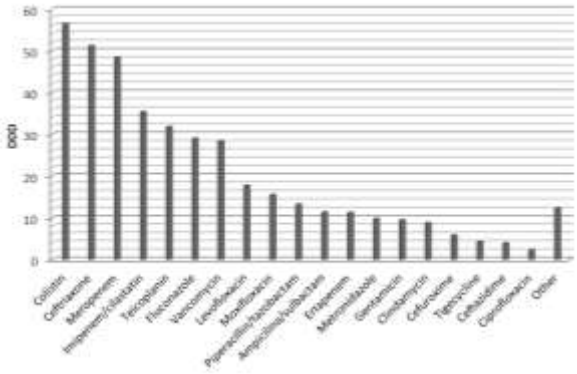

Fig.4. The antimicrobials used for the treatment of HAI. Legend: "Other": Chloramphenicol, Ceftaroline, Amoxicillin/clavulanic acid, Doxycycline, Trimethoprim/Sulfamethoxazole, Ampicillin, Ketoconazole etc.

We further observed the frequency of prescription for the major classes of antibiotics during the studied period in the TECCH -ICU. The most frequently used antibiotics were cephalosporins (236.95 DDD per 1000 patients / day), followed by carbapenems (193.58 DDD per 1000 patients / day) and glycopeptides (107.42 DDD per 1000 patients / day) (Figure 5).

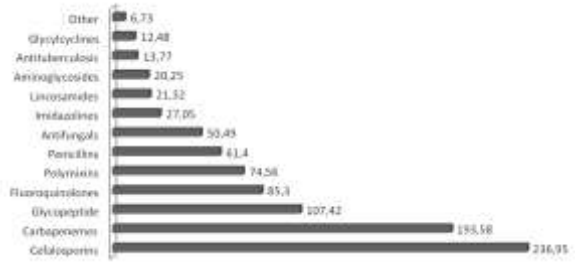

Fig.5. The most frequently prescribed antimicrobial groups in the ICU during the studied period, defined as DDDs per patient-days. Legend: "Other" antimicrobials include: aminophenols, macrolides, sulfonamides, tetracyclines, oxazolidindiones, monobactams.

DISCUSSION

The antibiotic consumption in a hospital department is correlated to the number of infections to which patients in that department are exposed. Also, the most frequently prescribed antibiotics depend upon the resistance profiles of microorganisms involved in the aetiology of infections.

Literature data show that the highest risk for acquiring multidrug resistant (MDR) germs occurs in patients admitted in ICUs, the risk being associated to several factors such as: prophylactic or curative antibiotic therapy, exposure to invasive diagnostic and treatment procedures, long term contact with healthcare personnel, long term hospitalization, as well as limited availability of space in the hospital department.

A study conducted in Bulgaria in 2003 revealed an over 10 times higher prevalence of HAI in ICU as compared to other hospital departments (13).

Numerous interventions addressing the improvement of antibiotic use have been described: restrictive antibiotic use programmes, the use of special forms limiting the number of therapeutic options, clinical guides. All these aim at a restrictive antibiotic use with consecutive reduced costs, together with decreasing the excessive prescription of wide spectrum antibiotics.

In our study, the four most frequently prescribed were wide spectrum antibiotic classes: cephalosporins, carbapanems, glycopeptides and fluoroquinolones, due to the specific ICU pathology and to the presence of MDR germs.

For prophylactic treatments, cephalosporins were by far the most frequently used antimicrobial class (62% second generation cephalosporins with 25.99 DDD per 1000 patient days and 38% third generation cephalosporins). On the other hand, for the treatment of underlying pathology, cephalosporins are very closely followed by carbapenems (96.66 DDD per 1000 patient days for cephalosporins and 88.92 DDD per 1000 patient days for carbapenems), and in HAI cephalosporins are exceeded by carbapenems which have the broadest antibacterial spectrum of the β-lactam class (largely because they are so β-lactamase stable) (14).

Due to their broad antibacterial spectrum covering gram-positive, gram-negative, and anaerobic bacteria, carbapenems are useful for the treatment of a wide variety of infections (14).

In a study conducted in Germany that included 35 ICUs, the authors observed that the pooled antibiotic usage density (AD=DDD/1000 patient days) was the highest for penicillins with lactamase inhibitor: 338.3 AD, followed by quinolones with 155.5 AD and second generation cephalosporins with 124.6 AD. The other classes in decreasing AD order were: third generation cephalosporins (109.5 AD), carbapenems (83.7 AD), extended spectrum penicillins (70.3 AD) and glycopeptides (42.3 AD). The ICUs that took part in this study were of different profiles: non-surgical, interdisciplinary and surgical (8).

Examining the results of a study conducted in France and published in 2002, starting with 1998, the antibiotic consumption in decreasing AD order may be observed: penicillins 981, cephalosporins 484, fluoroqinolones 272, aminoglycosides 216, glycopeptides 130, carbapenems 114, and other antibiotic classes, 114 DDD per 1000 patient-days, respectively. These results were obtained after implementation of an antibiotic-use policy (15).

In another study conducted in a German surgical ICU, that evaluated the impact of reducing the duration of antibiotic prophylaxis, the mean antimicrobial use density (AD) of antibiotic groups with an AD>30 was reported. Results have been compared with the pre-intervention level (January 2002-December 2003).Total antibiotic use was: 1035.9, cephalosporins - 423.5, amoxicillin – clavulanic acid - 129.8, penicillins with beta-lactamse inhibitior -129.5, quinolones - 129.2, carbapenems - 75.8, imidazoles - 72.3, piperacillin-tazobactam - 58.2, sulfonamides + trimethoprim 46.9 (16).

In another study published in 2009 and conducted in the same clinical department of TECCH, a marked yearly decrease in antibiotic use was observed (2004-2005). This was justified by the appointment in 2005 of the Antibiotic Therapy Commission in TECCH, with a strict policy on antibiotic treatments. In 2004, the most frequently used antibiotic classes were penicillins - 578.98, third generation cephalosporins – 280.77, penicillins with beta-lactamase inhibitors – 139.40, aminoglycosides – 76.23, fluoroquinolones - 20.83, carbapenems - 20.36, glycopeptides - 13.26 etc., penicillins being reduced to 7.75 (13).

CONCLUSION

The most frequently used classes of chemotherapeutic drugs were third generation cephalosporins, followed by the second generation, represented by cefuroxime alone, the fifth generation on the third position represented by ceftaroline and, finally, the fourth generation represented by cefpirome. These were used for prophylactic purposes and for treating underlying infectious pathology, while carbapenems were especially involved in treating HAI.

REFERENCES

1. Dereli N, Ozayar E, Degerli S, Sahin S, Koç F. Three-Year Evaluation of Nosocomial Infection Rates of the ICU. *Rev Bras Anestesiol.* 2013;63(1):73-84.
2. Emmerson M. Antibiotic usage and prescribing policies in the intensive care unit. *Intensive Care Med* 2000; 26: S26-S30.
3. Kollef MH. Optimizing antibiotic therapy in the intensive care unit setting. *Critical Care* 2001; 5:189-195.
4. Kaier K. Economic implications of the dynamic relationship between antibiotic use and hospital-acquired infections. *Value in Health* 2012; 15: 87-93.
5. Raineri E, Crema , Dal Zoppo S, Acquarolo A, Pan A, Carnevale G, Albertario F, Canadiani A. Rotation of antimicrobial therapy in the intensive care unit: impact on incidence of ventilator-associated pneumonia caused by antibiotic-resistant Gram-negative bacteria. *Eur J Clin Microbiol Infect Dis* 2010; 29:1015-24.
6. Roberts JA, De Waele JJ, Dimopoulos G, Koulenti D, Martin C, Montravers P, Rello J, Rhodes A, Starr T, Wallis SC, Lipman J. DALI: Defining antibiotic levels in intensive care unit patients: a multi-centre point of prevalence study to determine whether contemporary antibiotic dosing for critically ill patients is therapeutic. *BMC Infectious Diseases* 2012, 12:152
7. Tao L, Hu B, Rosenthal VD, Gao X, He L. Device-associated infection rates in 398 intensive care units in Shanghai, China: international nosocomial infection control consortium (INICC) findings. *International Journal of Infectious Diseases* 2011; 15: e774-e780.
8. Meyer E, Jonas D, Schwab F, Rueden H, Gastmeier P, Daschner FD. Design of a Surveillance System of Antibiotic Use and Bacterial Resistance in German Intensive Care Units (SARI). *Infections* 2003; 31: 208-215.
9. Knox KL, Homles AH. Regulation of antimicrobial prescribing practices-a strategy for controlling nosocomial antimicrobial resistance. *Int J Infect Dis* 2002; 6: S8-S13.
10. Klugman KP. Implications for antimicrobial prescribing of strategies based on bacterial eradication, *Int J Infect Dis* 2003; 7: S27-S3.
11. www.whooc.no
12. Hutchinson JM, Patrick DM, Fawziah M, Ng H, Bowie WR, Heule L, Muscat M, Monnet DL. Measurement of antibiotic consumption: A practical guide to the use of the anatomical therapeutic chemical classification and defined daily dose system methodology in Canada. *Can J Infect Dis* 2004; 15(1).
13. Voicu M. Corelatii intre antibiorezistenta unor tulpini bacteriene si consumul de chimioterapice antiinfectioase dintr-o sectie de Terapie Intensiva, 2009.
14. Mandell GL, Bennett JE, Dolin R. Principles and practice of Infectious Diseases, seventh edition; 2010
15. Geissler A, Gerbeaux P, Granier I, Blanc P, Facon K, Durand-Gasselin J. Rational use of antibiotics in the intensive care unit: impact on microbial resistance and costs. *Intensive Care Med* 2003; 29: 49-54.
16. Meyer E, Schwab F, Pollitt A, Bettolo W, Schroeren-Boersch B, Traumann M. Impact of a Change in Antibiotic Prophylaxis on Total Antibiotic Use in a Surgical Intensive Care Unit. *Infection* 2010; 38:19-24.

CONSUMUL DE ANTIBIOTICE ÎNTR-O SECȚIE DE TERAPIE INTENSIVĂ

REZUMAT

Pacienții internați într-o secție de terapie intensivă (ICUs) sunt expuși unui risc mai mare de a dobândi infecții intraspitalicești (HAI) comparativ cu alți pacienți spitalizați și pot deceda dacă infecția survine în asociație cu alți factori de risc. Pacienții internați în ICU sunt de obicei în stare critică și necesită monitorizări frecvente; rata infecțiilor variază în limite largi în funcție de tipul ICU.

Studiul s-a desfășurat în cadrul departamentului ICU al Spitalului Județean de Urgență Timișoara (TECCH), România. Datele au fost înregistrate pe o perioadă de 18 luni, între 01.01.2012 și 30.06.2013, pentru toți pacienții internați la ICU care au primit tratament antibiotic. Pentru fiecare pacient au fost monitorizați următorii parametri: vârsta, sexul, diagnosticul la internare, originea, evoluția, tipul și cantitatea de agenți antimicrobieni folosită (în cadrul ICU), precum și numărul zilelor de tratament pentru fiecare antibiotic.

Au fost colectate date referitoare la 1186 de pacienți ai ICU, cu privire la numărul și calea de administrare a agenților antimicrobieni, pacienții având vârsta medie de 61,78 ani (între 4 luni și 91 de ani). Pe baza sistemului de clasificare WHO am obținut un total de 911,24 DDD. Dintre cei 1186 de pacienți tratați cu antimicrobiene, care au fost studiați pe o perioadă de 18 luni în TECCH-ICU, 504 au primit tratament profilactic, 462 au fost tratați pentru patologie subiacentă, iar 220 de pacienți au fost tratați pentru infecții intraspitalicești.

Cele mai frecvent utilizate clase de medicamente chemoterapice au fost cefalosporinele de generația a treia, urmate de cele de generația a doua, reprezentate de monoterapie cu cefuroxima; generația a cincea s-a clasat pe locul trei și a fost reprezentată de ceftarolina, iar în final, generația a patra a fost reprezentată de cefpiroma. Aceste antibiotice au fost folosite în scop profilactic și pentru tratarea patologieie infecțioase subiacente, în timp ce carbapenemii au fost utilizați în special pentru tratarea HAI.

Cuvinte cheie: antimicrobiene, secții de terapie intensivă (ICUs), infecții intraspitalicești (HAI)