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PROF. DR. FRANCISC SCHNEIDER
PO BOX 135
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e-mail: carmen.tatu@umft.ro

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CUPRINS

THE EFFECTS OF *CORNUS MAS, PRUNELLA VULGARIS AND CRATAEGUS MONOGYNA* PLANT EXTRACTS ON VIABILITY OF NEONATAL RAT CARDIOMYOCYTES

BĂDĂLICĂ-PETRESCU MARIUS[1], TUDOR ANCA[2*], STOICHESCU-HOGEA GHEORGHE[3], SOCACIU CARMEN[4], DRĂGAN SIMONA[1]

[1]Department of Cardiology, University of Medicine and Pharmacy "Victor Babeș" Timișoara
[2]Department of Medical Informatics, University of Medicine and Pharmacy "Victor Babeș" Timișoara [3]City Hospital Timisoara
[4]University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Science and Technology, Cluj-Napoca

ABSTRACT

Aim: Our study aimed to evaluate the effects of three aqueous plant extracts rich in polyphenols (*Crataegus monogyna*, *Cornus mas* and *Prunella vulgaris*) on cardiomyocytes taken from newborn rats, in normoxic conditions.

Materials and methods: Hearts from 270 newborn Wistar rats(1-2 days) were prelevated and introduced in ice cold phosphate-buffered saline solution (PBS). The ventricles were separated, minced, and introduced in trypsin solution(0,25%) for 25-30 minutes. The resulted cell suspension was centrifuged for 15 minutes, at 2000 rpm and 4°C, then the infranatant was separated and mixed with Dulbecco's Modified Eagle Medium (DMEM). Several 96-well plates were prepared and introduced in the incubator for 72 hours(37°C, 95% O_2, 5 %CO_2). Using the Folin-Ciocâlteu method, we were able to determine the total polyphenol content of each plant extract. Starting from the original stocks, we prepared six consecutive dillutions using bidistilled water for each plant extract. The dillutions were used for treating the cells in normoxic conditions. The viability measurement was performed using FLUOstar Optima plate reader, after calcein and propidium iodide-digitonine staining.

Results and conclusions: Only *Cornus mas* extract showed statistically significant improvement in cell viability versus the control group. *Crataegus monogyna* and *Prunella vulgaris* extracts had no significant effect on cell viability.

Keywords: plant extracts, polyphenols, normoxia, viability

INTRODUCTION

For centuries, various plants have been used for preventing or treating diseases. The leaves and/or fruits of medicinal plants proved to be rich in active constituents like polyphenols or flavonoid glycosides, thus having a major impact on reducing the incidence of chronic diseases(1).

As *Burta et al.(2)* showed, extracts of *Crataegus* species, also known as "hawthorn", due to their high content in flavonoids, were used in traditional medicine for decades in preventing or treating moderate heart diseases. Many studies were conducted by *Ammon (3)* to explain the relationship between the composition and the cardiotonic activity of *Crataegus* species extracts.

A number of 23 active constituents such as phenolic acids, anthocyanins and flavonoid glycosides were identified in *Crataegus monogyna* fruits (4).Therefore, due to its high concentrations of phenols, phenolic acids and proven in vitro antiradical activity, the *Crataegus monogyna* extract was evaluated as possible source of antioxidant phytochemicals (5).

Extracts obtained from *Crataegus* leaves and flowers using ethyl acetate were found to have the highest phenolic content (343.54 mg of gallic acid equivalents/g) and the highest DDPH radical scavenging activity. Worldwide known for its medicinal uses, the phytopharmaceutical characteristics of *Crataegus oxycantha* leaves, flowers, berries and bark were recently rewied (6).

Cornus mas, also known as "Cornelian cherry", is a medicinal plant used for its cardiotonic and anticancer effects, as well as for treating inflammation (7).

The chemical characterization of *Cornus mas* fruits showed high amounts of phenolic and antioxidant compounds. Flavonoids, anthocyanins, vitamins E, C, thiamine (B1) and riboflavin (B2) were also found in its fruits (8). A recent study revealed the cardioprotective effect of *Cornus mas* fruit extract against cardiotoxicity induced by carbon tetrachloride (CCl4) in a rat model (9).

The antioxidant activity of Cornus mas fruits was observed during antioxidant assays in Turkey (10, 11), in Greece (12) and in other countries (13, 14). The DPPH scavenging activity was also high and shown to be increased by salycilic acid treatment due to significant increase of the total phenols, flavonoids, anthocyanins and ascorbic acid content (15).

The antioxidant capacity expressed as DPPH scavenging effect and the total phenolics expressed in gallic acid equivalents in both *Crataegus monogyna* and *Cornus mas* extracts (mg GAE/100 ml) were demonstrated in another study (16).

Once used in traditional medicine from all around Europe and Asia for treating fever, wounds and throat infections (17), *Prunella vulgaris* is now a rediscovered plant due to its potential uses in medicine. Its compounds can be used as antibacterial, antibiotic and antiseptic, as well as antipyretic, diuretic and hypotensive agents (18, 19).

Received 17th of March 2014. Accepted 20th of May 2014. Address for correspondence: Tudor Anca, Department of Medical Informatics, "Victor Babes" University of Medicine and Pharmacy Timisoara, Eftimie Murgu Square No. 2A, RO-300041, Timisoara, Romania, phone/fax: +40256490288; e-mail: atudor@umft.ro.

Using High Performance Liquid Chromatography (HPLC) analysis, it was possible to identify the main compounds in *Prunella vulgaris* i.e. phenols like quercetin, rutin, caffeic and rosmarinic acid (20). The rosmarinic acid found in *Prunella vulgaris* extract was correlated with the cardioprotective effect against oxidative stress induced in rats (21). Furthermore, the effect on eNOS gene expression of the *Prunella vulgaris* extract resulted in favourable influences on blood vessels. By acting as an eNOS upregulating agent, the extract proved to have a promising cardioprotective potential (19).

Crataegus monogyna, *Cornus mas* and *Prunella vulgaris* are three medicinal plants well known for their cardiotonic effect. Rich in polyphenols, the antioxidant qualities of these plants is unquestionable.

Our study aimed to compare the effects of these plant extracts on neonatal rat cardiomyocytes used in cell culture, while being subjected to normoxic conditions.

MATERIALS AND METHODS

Aqueous plant extracts preparation

Only fresh leaves of *Crataegus monogyna* and *Cornus mas* and inflorescences of *Prunella vulgaris* were harvested from Cluj-Napoca Botanical Garden in June 2012. To prepare the extracts, 15 g of each plant were infused in 100 ml bi-distilled water in a sonication bath for 30 minutes, then centrifuged at 2000 rpm for 10 minutes. Before performing HPLC-DAD-ESI (+)-MS analysis, the supernatant was collected and filtered through a 0,45 µm filter.

Total polyphenols determination

Total phenolic content was determined using Folin-Ciocalteu method, adapted by Singleton (1999). 1 ml filtered extract of each plant was mixed with 5 ml Folin reagent, homogenized, then 15 ml Natrium carbonate 7,5% were added to the mixture. The absorbance of the solution was observed after 2 hours, at λ= 750 nm. All results were expressed in milligrams of gallic acid equivalents (GAE)/100 ml extract.

The highest quantity of polyphenols was found in *Cornus mas* extract (105,03 mg GAE/100 ml), followed by *Prunella vulgaris* (39 mg GAE/100 ml) and *Crataegus monogyna* (31,5 mg GAE/100 ml).

Cardiomyocyte isolation and culture

All the experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (22) and were approved by the Ethics Committee of University of Medicine Szeged, Hungary.

The isolation protocol was performed on 1-2 days newborn Wistar rats, in accordance with a method described by Gorbe et al. (23).

Hearts from 270 newborn Wistar rats were excised and placed in ice-cold phosphate-buffered saline solution (PBS) pH 7.2. The ventricles were separated from the atria, minced using forceps and washed three times with sterile PBS to rinse away tissue debris and erythrocytes.

For dissociation into single cells, the cut pieces of the ventricles were collected in 0.25% trypsin solution and introduced in 37°C water bath for 30 minutes (24, 25). After the digestion period, the cell suspension was centrifuged for 15 minutes, at 2000 rpm and 4°C.

The supernatant was removed using 5 ml Pasteur pipettes. The cell pellet was re-suspended in Dulbecco's modified Eagle's medium (DMEM I), which was supplemented with 1% L-Glutamine, 1% Antibiotic and Antimycotic mixture and 10% fetal bovine serum (FBS). The resulted cell suspension was then collected in Falcon tubes and diluted in 4-5 ml DMEM I.

We performed the pre-plating stage by adapting the method described by Blondel et al. in 1971(24). Thereby, the non-muscular cells were eliminated after the cell suspension was plated onto 6-well plates at 37°C for 90 min.

After the pre-plating period finished, the suspension containing non-adherent myocytes was collected in Falcon tubes and agitated.

The cell counting was performed manually using a Burker chamber and an inverted microscope. Only the round-shaped medium-sized cells from 3 big squares were counted and the average was took into consideration for further calculations.

In order to obtain a 75-80% confluence, the cell suspension was homogenized using a pipette and plated at a chosen density of $2x10^4$/well on non-mobile 96-well plates. Each well was supplemented with 100 µl DMEM I and the plates were gently shaked horizontally and vertically for better distribution of the cells, before being placed into the incubator.

The plates were left for 24 hours in an incubator, being subjected to a humidified atmosphere at 37°C, 95% air and 5% CO_2.

After the first incubation period finished, the DMEM I medium was substituted with DMEM II (Dulbecco's modified Eagle's medium with 1% fetal bovine serum (FBS), 1% L-glutamine and 1% Antibiotic-Antimycotic mixture).

Using the inverted microscope, the contractile activity of the cardiomyocytes was checked at 24, 48 and 72 hours.

The normoxic experiments started 72 hours after plating.

Preparation of normoxic control solution

As described by X Li *et al* (26), to obtain the normoxic solution, we used the following (Table I):

Table I. Normoxic solution. *The $MgCl_2$ saturated solution contains 0,3774 g/1 ml.*

NORMOXIC SOLUTION				
	MW	mM	Mili-grames/100 ml	Grames/ 1 L
NaCl	58.4	125	730	7.305
KCl	74.5	5.4	40.2	0.402
NaH_2PO_4.1 H_2O	137.9	1.2	16.6	0.1655
$CaCl_2$. 2 H_2O	174.02	1	17.4	0.174
$MgCl_2$ x 6 H_2O (saturated solution)	95.2	0.5	12.6 UI	126 UI
Glucose	198.1	15	297.2	2.972
Taurine	125.1	5	62.6	0.625
Creatine monohydrate	149.1	2.5	37.2	0.372
HEPES	238.3	20	476.6	4.766
BSA		0.1%	100	

Before using the normoxic solution, the pH was set to 7.4.

During the experiments, a dilution of 1:1000 was obtained by mixing 1 µl bi-distilled water + 999 µl normoxic solution. This served as a control solution, it's effects on cardiomyocytes being compared with the effects of the three plant extracts.

Preparation of the *Cornus mas*, *Crataegus monogyna* and *Prunella vulgaris* dillutions

As we have shown earlier in another study (27), after applying the Folin-Ciocalteu method, the total polyphenols content for each plant extract was determined (Table II):

Table II. Total polyphenols content for *Cornus mas, Crataegus monogyna* and *Prunella vulgaris*

Extract sample	Mg GAE/100 ml
Cornus mas	105.03
Crataegus monogyna	31.5
Prunella vulgaris	39

Because of the different content in polyphenols, the initial extract stocks of all three plant extracts had to be standardized to a desired concentration of 0,1 mg polyphenols/ml solution, using bi-distilled water.

Starting with the original stocks, we have obtained six consecutive dilutions for each extract, as follows: 0.01 mg polyphenols/ml, 0.001 mg polyphenols/ml, 0.1 µg polyphenols/ml, 0.01 µg polyphenols/ml, 0.001 µg polyphenols/ml and 0.0001 µg polyphenols/ml (Table III).

Table III. *Cornus m., Crataegus m.* and *Prunella v.* dilutions (mg polyphenols/ml solution); *pps= polyphenols*

Cornus mas dilutions	**CO1** 0.01 mg pps/ml	**CO2** 0.001 mg pps/ml	**CO3** 0.1 µg pps/ml	**CO4** 0.01 µg pps/ml	**CO5** 0.001 µg pps/ml	**CO6** 0.0001 µg pps/ml
Crataegus monogyna dilutions	**CR1** 0.01 mg pps/ml	**CR2** 0.001 mg pps/ml	**CR3** 0.1 µg pps/ml	**CR4** 0.01 µg pps/ml	**CR5** 0.001 µg pps/ml	**CR6** 0.0001 µg pps/ml
Prunella vulgaris dilutions	**PR1** 0.01 mg pps/ml	**PR2** 0.001 mg pps/ml	**PR3** 0.1 µg pps/ml	**PR4** 0.01 µg pps/ml	**PR5** 0.001 µg pps/ml	**PR6** 0.0001 µg pps/ml

For preparing the dilutions, we used bi-distilled water obtained in the laboratory using the **Millipore Direct-Q3** water distiller.

Normoxic experiments

For the normoxic experiments, we used a protocol adapted after Gorbe A. et al. (23) and X Li et al. (26) which involved treatment of the cells with the desired dilutions of the plant extracts and incubation for 4 hours at 37°C, 5% CO_2, 95% air. After the 4 hours incubation, we changed the applied treatment with DMEM I and we let the cells for another 2 hours, in the same conditions (37°C, 5% CO_2, 95% air), in the incubator.

The protocol used for normoxic experiments is expressed in Figure 1:

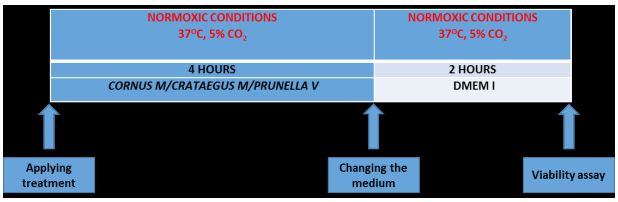

Fig.1. Working protocol for the normoxic experiments

The plating for each of the three plant extracts was performed after a fixed scheme, as shown in Figure 2:

Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9	Row 10	Row 11	Row 12
DMEM I	Norm ctrl+veh	PE 1	PE 2	PE 3	PE 4	PE 5	PE 6	Norm ctrl+ veh	Norm ctrl+ veh	DMEM I	No cells

Fig. 2. Plating example for 96-well plates. PE= plant extract dilution; Norm ctrl+veh= normoxic solution + bi-distilled water (1:1000)

Treatment with Cornus mas extract. The six dilutions obtained (see Table No. 3) were used for treating six rows of cells on each 96-well plate (from row 3 to row 8). We used 100 µl/well, at a dilution of 1:1000 (1 µl stock + 999 µl normoxic solution).

The same dilution was used for obtaining the Normoxic control+vehicle (1 µl bi-distilled water + 999 µl normoxic solution), which was applied over rows 2, 9 and 10, whereas rows 1 and 11 were left untreated, and row 12 had no cells to begin with.

The *Cornus mas* dilutions and the Normoxic control+vehicle solution were applied over the cells just before the 4 hours incubation period started. After the first incubation period finished, we changed the solution of each row with DMEM I and the plates were left for another 2 hours in the incubator.

Treatment with Crataegus monogyna and Prunella vulgaris extracts. We followed the same steps as in *Cornus mas extract* treatment.

Viability assay

For the viability assessment, we used the calcein/propidium iodide (PI)-digitonine staining and the FLUOStar Optima plate reader. Both calcein and PI-digitonine stocks were prepared in our laboratory, half an hour before the viability assay started.

The viability assessment using calcein is based on the conversion of the cell permeant non-fluorescent calcein AM dye to the fluorescent calcein dye due to intracellular esterase activity in living cells (27). Propidium iodide (PI) is membrane impermeant, therefore it is not usually found inside viable cells with intact membranes. However, when it is applied over dead cells, it penetrates the cellular membrane and gains access to the nucleic acids, and the fluorescent signal increases (28).

After the last incubation period which lasted for 2 hours, the plates were removed from the incubator and the cells were gently washed three times using 200 µl Dulbecco's Phosphate Buffered Saline solution (DPBS)/well.

When the washing was finished, 3µM calcein AM was added

over the cells, 200 µl/well and the plates were left for 30 minutes at 37°C in a dark room. The 3µM calcein AM was changed with 200 µl DPBS /well and the plates were introduced in the plate reader to evaluate the live cells.

Having evaluated the percentage of living cells, the plates were removed from the plate reader, the DPBS was removed and 2.5-5 µM PI-digitonine was added over the cells, 200 µl/well. The plates were left again for 30 minutes, at 37°C in a dark room, then the Pi-digitonine dye was replaced with 200 µl DPBS /well and viability measurement using the plate reader followed.

All data was collected and interpreted by the FLUOStar Optima plate reader, then transposed on Excel working sheets.

Statistical analysis

The results have been statistically analyzed and expressed in the form of mean ± SD. Mann-Whitney nonparametric test and SPSS15 software were used to examine the results and to compare the mean values for different experimental groups. A value of $P < 0.05$ was considered as significant. The graphs were realized using Excel software.

RESULTS

Cornus mas vs Normoxic control+vehicle

All six dilutions of *Cornus mas* extract proved to have a significant effect in increasing the cell viability vs Normoxic control+vehicle group ($p<0.05$).

The best increase in cellular viability was observed when treating the cells with CO1 (0.01 mg pps/ml) ($p= 0.012$) and CO3 (0.1 µg pps/ml) ($p= 0.001$) dilutions.

Prunella vulgaris vs Normoxic control+vehicle

None of the six dilutions of *Prunella vulgaris* extract used for treating the cells had a significant effect in increasing the viability vs *Normoxic control+vehicle* treatment ($p=0.05$).

Moreover, the PR1 dilution proved to be toxic and killed all the cells.

Crataegus monogyna vs Normoxic control+vehicle

Although the cardioprotective effect of *Crataegus spp.*extracts used in hypoxic condition was demonstrated (29), none of the six dilutions used for treating cells in normoxic conditions had any effect in increasing cell viability.

Cornus mas vs Crataegus monogyna vs Prunella vulgaris

Using the Mann-Whitney non-parametrical test, the dilutions of the three plant extracts were compared.

Cornus m. vs Prunella v. : after comparing each dilution (CO1 vs PR1, CO2 vs PR2, etc.) the *Cornus mas* extract was found to be significantly better than *Prunella vulgaris* extract in increasing cell viability when used in normoxic conditions. The p values are expressed in Table IV.

Table IV. *Cornus m. vs Prunella v.*- Mann-Whitney nonparametric test

Dilutions	CO1 vs PR1	CO2 vs PR2	CO3 vs PR3	CO4 vs PR4	CO5 vs PR5	CO6 vs PR6
p	<0.001	0.005	0.004	<0.001	<0.001	0.004
α	0.001	0.01	0.01	0.001	0.001	0.01

Cornus m. vs Crataegus m. : the *Cornus mas* dilutions proved to have a significant effect in increasing cell viability vs *Crataegus m.* dilutions, as expressed in Table V.

Table V. *Cornus m. vs Crataegus m.*- Mann-Whitney nonparametric test

Dilutions	CO1 vs CR1	CO2 vs CR2	CO3 vs CR3	CO4 vs CR4	CO5 vs CR5	CO6 vs CR6
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
α significance level	0.001	0.001	0.001	0.001	0.001	0.001

Crataegus m. vs Prunella v.: as shown previously, none of the two plant extracts had a significant effect in increasing cell viability vs *Normoxic control+vehicle* treatment. Still, after comparing their dilutions, a significant difference was observed. Except for PR1 dilution, all *Prunella v.* dilutions were significantly superior compared with *Crataegus m.* dilutions (Table VI).

Table VI. *Prunella v. vs Crataegus m.*- Mann-Whitney nonparametric test

Dilutions	PR1 vs CR1	PR2 vs CR2	PR3 vs CR3	PR4 vs CR4	PR5 vs CR5	PR6 vs CR6
p	<0.001	<0.001	0.002	<0.001	0.019	<0.001
α significance level	0.001	0.001	0.01	0.001	0.05	0.001

A schematic representation of the effect of the three plant extracts and *Normoxic control+vehicle* treatment on neonatal rat cardiomyocytes can be seen in Figure 3.

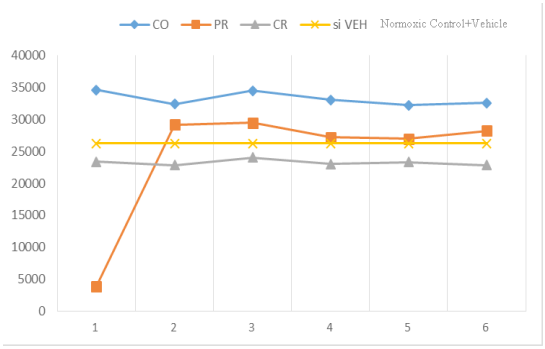

Fig. 3. Comparison between mean values of each dilution of plant extract and Normoxic Control+Vehicle solution

DISCUSSIONS

The effect of three plant extracts on neonatal rat cardiomyocytes used in cell cultures was studied. As proven, only the *Cornus mas* dilutions had a significant effect in increasing cell viability, while being applied in normoxic conditions vs *Normoxic control+vehicle* treatment ($p<0.05$)

The fact that *Cornus mas* plant extract was found to have the highest content in polyphenols (see Table II) is not correlated with the increase of cell viability because all three plant extracts were standardized and a concentration of 0,1 mg polyphenols/ml solution was used for the initial stocks. This allows the

speculation that it is not the quantity, but indeed the quality of polyphenols that might be correlated with the increase of cell viability in normoxic conditions.

On the other hand, after performing HPLC-DAD-ESI (+) MS analysis in a previous study, we have shown that these three plant extracts contain a lot of other constituents like flavonoids and flavonoid derivates that are well known for their antioxidant capacity (16).

The most complex plant extract was represented by *Cornus mas*, followed by *Prunella vulgaris* and *Crataegus monogyna* extracts.

Cornus mas extract contained a mixture of flavonoid glycosides and phenolic derivatives, represented mainly by epi-catechin, quercetin, kaempherol and derivatives, caffeic acid and derivatives of coumaric acid. *Prunella vulgaris* was foud rich in rosmarinic acid, rutin, betulinic and oleanolic acids, ursolic acid and tannins (30), while *Crataegus monogyna* extract contained mainly vitexin isomers like 2' and 4' –O-rhamnoside (16).

As for the antioxidant activity of each extract, the highest stability and potential of flavonoids to express scavenging potential against DPPH in hydrophilic environment was shown by the *Cornus mas* extract (16).

Therefore, the antioxidant activity of *Cornus m.* extract can be attributed to polyphenols like epi-catechin and flavonoids like quercetin, two proven antioxidant agents that were studied before, both in *in-vitro* and in *in-vivo* experiments (31, 32).

Rosmarinic acid seems to be involved in conferring antioxidant capacity to the *Prunella v.* extract, as shown in a study conducted by R. Rasool (30).

After performing the HPLC-DAD-ESI (+) MS analysis that revealed the content of each plant extract and knowing the effects of each compound, it is easy to understand why the *Crataegus monogyna* extract was less effective in increasing cell viability.

Although the antioxidant effect of *Crataegus monogyna* and *Crataegus oxycantha* was demonstrated in numerous studies, we could not reproduced it. This failure could be due to the fact that we have used only aqueous extracts, instead of the alcoholic extracts. As proof stands the fact that there was a major difference between the compounds found in the two types of extracts. Therefore, while the aqueous extract of *Crataegus m.* contained mainly vitexin isomers like 2' and 4' –O-rhamnoside (16), the alcoholic extract was rich in proanthocyanidin, vitexine-2-O-rhamnoside, hyperoside, anthocyanin, chlorogenic acid and epicatechin (33).

Leaving aside the antioxidant capacity of each compound that can be isolated from the plant extracts that we have studied, an important role in increasing the cell viability could be played by the synergic effect of all compounds and their interactions.

Another important aspect is represented by the fact that obtaining an increase in cell viability is a matter of dose dependence. Therefore, further studies are needed in order to elucidate the appropriate dose, as well as the exact compounds and mechanisms that could be correlated with the viability increase.

CONCLUSIONS

In our experiments, all dilutions of *Cornus mas* extract had a significant effect in increasing the cell viability vs Normoxic control+vehicle group ($p<0.05$). The best increase was observed when the cells were treated with CO1 dilution (0.01 mg pps/ml) ($p= 0.012$), respectively CO3 dilution (0.1 µg pps/ml) ($p= 0.001$).

Neither *Prunella vulgaris,* nor *Crataegus monogyna* dilutions used for treating the cells, had a significant effect in increasing the viability vs *Normoxic control+vehicle* treatment ($p\geq0.05$).

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EFECTELE EXTRACTELOR DE PLANTE DIN *CORNUS MAS, PRUNELLA VULGARIS* SI *CRATAEGUS MONOGYNA* ASUPRA VIABILITATII CARDIOMIOCITELOR NEONATALE DE SOBOLAN

REZUMAT

Scop: Studiul nostru isi propune evaluarea efectelor a trei extracte apoase bogate in polifenoli (*Crataegus monogyna*, *Cornus mas* si *Prunella vulgaris*) asupra cardiomiocitelor recoltate de la sobolani nou-nascuti, in conditii de normoxie.

Materiale si metode: Au fost prelevate inimile a 270 de sobolani Wistar nou-nascuti (1-2 zile) si au fost introduse in solutie salina rece (PBS). Au fost indepartati ventriculii si fragmentati, iar piesele maruntite au fost introduse in solutie de tripsina (0,25%) timp de 25-30 minute. Suspensia celulara rezultata a fost centrifugata 15 minute, la 2000 rpm si 4°C, sedimentul fiind amestecat cu mediul de cultura Dulbecco's Modified Eagle Medium (DMEM). Au fost pregatite placi de cultura cu 96 de godeuri si introduse in incubator timp de 72 de ore (37°C, 95% O_2, 5 %CO_2). Folosind metoda Folin-Ciocâlteu am determinat continutul total de polifenol al fecarui extract de plante. Pornind de la solutiile stoc originale, am preparat sase dilutii succesive, folosind apa bidistilata pentru fiecare extract de plante. Dilutiile au au fost folosite pentru tratarea celulelor in conditii de normoxie. Au fost efectuate masuratori ale viabilitatii celulare folosind FLUOstar Optima plate reader, dupa marcarea cu iodura de propidiu-digitonina.

Rezultate si concluzii: Doar extractul de *Cornus mas* a indus o crestere a viabilitatii celulare semnificativa statistic comparativ cu grupul de control. Extractele de *Crataegus monogyna* si *Prunella vulgaris* nu au indus efecte semnificative asupra viabilitatii celulare.

Cuvinte cheie: extracte de plante, polifenoli, normoxie, viabilitate

SIMULTANEOUS LAPAROSCOPIC APPROACH OF COLORECTAL CANCER AND OF SYNCHRONOUS LIVER METASTASES – INITIAL EXPERIENCE

BREBU DAN, PANTEA STELIAN, LAZAR CAIUS, TARȚA CRISTI, DUȚA CIPRIAN, LAZAR FULGER
Surgery Department, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania
Second Surgery Clinic of Timisoara County Hospital

ABSTRACT

The simultaneous resection of colorectal cancer (CRC) and of synchronous liver metastases is a debate topic related to the post-operative morbidity as compared to the staged resection. The purpose of this study is to analyse the results found in a series of eight patients with CRC and synchronous liver metastases who benefitted from combined colorectal and liver laparoscopic surgery. A prospective assessment was performed in terms of patients' characteristics, tumours' characteristics, operative variables and postoperative results. The primary tumour was localized at the level of the colon in six patients, and at the level of the rectum in two patients, while the synchronous liver metastases were predominantly solitary. A laparoscopic approach was applied to all 8 patients, who underwent colorectal resection (R0), as well as metastasectomies and hepatic wedge resections. There was no need to convert to classic surgery. The sample was extracted by pararectal incision or by transverse suprapubic incision. The median operative time was approximately 313 minutes (range 151-394 minutes), with an average blood loss of 600 ml (range 200-850 ml).The postoperative length of stay varied between 6 and 14 days. On the basis of this initial experience of a single centre, the laparoscopic simultaneous resection of colorectal cancer and the hepatic resection can be performed on the selected patients suffering from CRC and SLM, with satisfactory short-term results.

Key words: colorectal cancer, liver metastases, laparoscopic surgical approach

INTRODUCTION

Liver is the most frequent site of hematogenous dissemination of colorectal cancer (CRC) (1, 2). Liver metastases are present in 10-25% of the patients during the surgical intervention for colorectal malignancy (3).The surgical resection is the most efficient and potentially curative treatment for the liver metastases of CRC (4).The treatment strategies and the results for these patients developed and underwent several changes due to the technical innovations (5-6).Laparoscopic surgery improves the accuracy of the surgical approach, the postoperative recovery, it minimizes the postoperative pain, reduces the rate of abdominal wall infection, decreases the hospital length of stay, allows a more rapid return to normal activities, has better cosmetic results and it does not compromise the oncologic result (7,8).

There are several treatment options for the patients suffering from CRC with synchronous liver metastases (SLM), depending on the location of the primary tumour (rectum or colon) and on the extent of liver disease. The controversies are related to the concept that the staged resection of the primary tumour and of the liver metastases is better tolerated by the patient. Recently, a systemic analysis has shown that synchronous colorectal and hepatic resection led to a shorter hospital stay and to fewer complications, as compared to a staged resection (9).The specialised literature shows that the staged laparoscopic resection, initially of the primary tumour and subsequently of the hepatic lesions is more documented than the combined laparoscopic resection of CRC and SLM, for which there are only a few references.

The purpose of this study is to assess the initial experience of the simultaneous laparoscopic resection of CRC and SLM and to monitor the evolution and the prognosis of these cases on the short term.

MATERIAL AND METHODS

Between January 2012 and March 2013 the Second Surgery Clinic of Timisoara County Hospital had 48 laparoscopic surgery cases for patients suffering from CRC, of which 15 also suffered from SLM. Of the 15 patients, 8 patients with CRC and SLM were selected to benefit from combined laparoscopic colorectal and liver surgery. The other 7 patients required major hepatectomy and they were excluded from the study. The data were prospectively collected and analysed for this study. The assessed parameters were the following: the patients' characteristics, the tumours' characteristics, the operative variables and the postoperative results.

Multimodal treatment of CRC and SLM

For the patients suffering from CRC and SLM, the decision for the best treatment was established depending on the location of the primary tumour, the complexity of the primary tumour resection, the location of the liver metastases, as well as their size and number, the feasibility of the laparoscopic approach and the general condition of the patient. For the series under study we performed simultaneous resection in patients undergoing colorectal resection with anastomosis in elective settings (T1-T3) and a single or multiple liver lesions (up to 3 lesions) with a peripheral location on segments 2-6 which allowed a limited hepatic resection.

Neoadjuvant therapy was administered for the primary rectal tumours (2 patients), consisting of short-course radiotherapy (5 x 5 Gy), followed by 3 to 5 systemic chemotherapy cycles (capecit-

Received 12th of March 2014. Accepted 15th of May 2014. Address for correspondence: Dan Brebu, MD, PhD Student Department of Surgery, University of Medicine and Pharmacy "Victor Babeș" Timișoara, Iosif Bulbuca Street, No. 10, Timișoara, România; Phone: +40755012719, e-mail: dr.brebudan@gmail.com

abine). All the cases have been analysed by a multidisciplinary team, the treatment being adapted to each particular case and well argumented. The protocol of our clinic was respected in terms of laparoscopic approach. The other patients underwent classic surgery or a staged approach.

Surgical approach

The laparoscopic approach of colorectal tumours was a medial-to-lateral approach. A right pararectal incision was performed for the right hemicolectomy in order to extract the resection specimen, as well as to perform an extracorporeal anastomosis. A Pfannenstiel incision was performed on the left colon in order to extract the resection specimen, followed by an intracorporeal colorectal anastomosis with circular stapler. The laparoscopic liver resection was performed by the surgeon standing between the legs of the patient. Hepatic wedge resections were performed for the peripheral metastases and metastasectomies. For the laparoscopic liver resection, 2-3 additional trocars were mainly used, as compared to the ones necessary for the approach of the colorectal primary tumour. The 10 mm trocar situated at the level of the umbilicus to ensure the view, initially used for the approach of the primary lesion, is used for the subsequent approach of the liver lesions. 2 additional trocars of 5 mm are placed in the upper abdomen, left and right. When needed, an additional 5 mm trocar was used.

Enseal® advanced technology instruments (advanced bipolar technology) or LigaSure™ instruments were used for the transection of the hepatic parenchyma, and Tachosil® *absorbablefibrin* sealant patch was used for the additional hemostasis of the hepatic section surface. The hepatic resection specimen was placed in a plastic bag and extracted through the pararectal incision and the Pfannenstiel incision, respectively.

RESULTS

6 men and 2 women diagnosed with CRC and SLM were included in the study. The preoperative diagnosis of CRC and SLM on the basis of the investigations performed was assigned to 6 patients. During the laparoscopic surgery, two patients were diagnosed with SLM in segments 2 and 6 and they were included in the protocol for simultaneous resection. The average age was 68 years (range 54-76 years). The characteristics of each patient are shown in Table I. The average body mass index was 29.0 (range 24.9-31.2) kg/m^2. A patient suffered an emergency laparoscopic cholecystectomy 2 months before, for a phlegmonouslithiasic acute cholecystitis. During laparoscopy, a hepatic lesion was identified and subsequently investigated. The colonoscopy revealed a tumour in the sigmoid colon. The preoperative treatment of the rectal cancer consisted of a short-course radiotherapy (5 x 5 Gy) and an associated systemic chemotherapy (capecitabine). All the 8 laparoscopic resections were successfully completed. There were no conversions to classic surgery. The surgical results are shown in Table II.

The following procedures have been performed: laparoscopic right hemicolectomy and wedge resection of segment 2/3 with extraction of the specimens through the right pararectal incision, laparoscopic sigmoidectomy with metastasectomies in segments 3, 4, and 6 with extraction of the specimens through the Pfanennstiel incision, rectal anterior resection and metastasectomies in segments 3 and 6 with extraction of the specimens through the Pfanennstiel incision. The incision used to extract the specimens varied between 5 and 10 cm.

Table I. Patient characteristics and preoperative data

Patient Number	Sex	Age	Medical history	Primary tumor location	Hepatic metastasis location	No. of metastasis	Neoadjuvant treatment
1	M	63	Apendectomy Hypertension	Sigmoid	Segment 6	1	No
2	M	54	None	Sigmoid	Segment 3	1	No
3	M	72	Hypertension Anemia	Cecum	Segment 2/3	3	No
4	F	68	Acute cholecistytis Laparoscopic cholecystectomy	Sigmoid	Segment 2	1	No
5	M	77	Hypercolesterolemia Coronary heart disease	Ascending colon	Segment 3	1	No
6	F	70	Angina pectoris Hypertension Hypothyroidsm	Rectum	Segment 6	1	5x5 Gy
7	M	67	Apendectomy	Rectum	Segment 3	1	5x5 Gy
8	M	69	Hypercolesterolemia Atrial fibrillation	Ascending colon	Segment 2	2	No

Table II. Surgical results

Patient number	Type of operation	Operation time (min)	Blood loss (ml)	Postoperative hospital stay (days)	Resumption of bowel (hours)
1	Sigmoidectomy and metastsectomy of segment 6	275	650	8	36
2	Sigmoidectomy and metastasectomy of segment 3	181	200	6	30
3	Right hemicolectomy and atipicrezection of segments 2/3	230	500	9	48
4	Sigmoidectomy and metastasectomy of segment 2	260	450	8	36
5	Right hemicolectomy and metastasectomy of segment 3	190	550	14	72
6	Rectal anterior resection and metastasectomy of segment 6	354	600	9	48
7	Rectal anterior resection and metastasectomy of segment 3	315	750	8	48
8	Right hemicolectomy and metastasectomy of segment 2	210	350	10	72

The median operative time was 280 minutes (range 181-354 minutes), with a total estimated blood loss of 600 ml (range 200-750 ml).The intraoperative complications consisted of the bleeding at the level of the section surface, solved with suture with Prolene 4-0 and by the introduction of an additional trocar. A patient showed a Pfannenstiel wound infection, and 2 patients suffered from delayed gastric emptying that required a nasogastric tube. Other postoperative complications: a patient developed a lower urinary tract infection and hypertension episodes that were managed by the administration of antibiotics and antihypertensives. The average hospital stay was 9 days, ranging between 6 and 14 days. There were no records of postoperative mortality. The R0 resection of the primary tumour and of the hepatic lesions was performed to all patients. Details regarding the pathological examination are shown in Table III.

Table III. Pathological examination

Patient number	p/yTN stage	Radicality	Diameter SLM (cm)	Resection margin SLM (mm)
1	pT2N1	R0	1.5	6
2	pT3N0	R0	2.4	8
3	pT2N2	R0	2/1.5/0.9	>10
4	pT3N0	R0	1.8	8
5	pT2N1	R0	1.5	7
6	yT1N1	R0	1.3	5
7	yT2N0	R0	2	6
8	pT2N2	R0	2.1/1.6	5

DISCUSSION

The advantages of the laparoscopic approach in colorectal surgery or the hepatic resections performed by experienced surgeons (8) led to a significant increase in the number of such interventions during the last years. The separate laparoscopic approach of colorectal cancer and of colorectal cancer metastases was proven and resulted in a more rapid recovery and a low morbidity rate, with oncologic results similar to those obtained by open surgery (7,8). This suggests that a combined laparoscopic approach both for the primary tumour and for the liver metastases may be beneficial for patients who candidate for a simultaneous resection.

Moreover, the open surgery for the resection of the colorectal primary tumour and of synchronous metastases may require an extended incision, which may sometimes be lacerating, especially when the tumours have opposite location (for instance the rectum and the right liver lobe).Using the laparoscopic approach, the exposure can be improved even when from an anatomical point of view there is a narrow pelvis and sites to which access is difficult, in the upper abdominal area. The feasibility of the simultaneous laparoscopic approach was proven by our initial experience in 8 cases, and the results obtained confirm the data found in the specialised literature for this topic, presenting series of a comparable size (Table IV).

Table IV. NR: not reported, LH: left hemihepatectomy, LLS: left lateral sectionectomy, M: metastasectomy, S: segmentectomy, RH: right hemihepatectomy, RFA: radio frequency ablation, *13 resections in 10 patients.

Author	Year	Nr. of patients	Laparoscopic liver resection (type)	Time (min)	Blood loss (ml)	Postoperative hospital stay (days)
Geiger et al. [10]	2006	1	LLS	330	600	4
Leung et al. [11]	2006	1	LLS	350	500	7
Vibert et al. [12]	2006	8	NR	NR	NR	NR
Law et al. [13]	2008	4	NR	NR	NR	NR
Bretagnol et al. [14]	2008	1	1 LLS 2 M	NR	NR	NR
Pessaux and Panaro [15]	2009	3	1 M + RFA	NR	NR	NR
Sasaki et al. [16]	2009	9	2 LLS	418	219	9
			7 M	(215–520)	(32-745)	(7-26)
Casaccia et al. [17]	2010	1	1 LLS	455	NR	12
Lee et al. [18]	2010	10*	6 LLS	401	500	10
			5 M	(230-620)	(60-1000)	(7-15)
			1 S	NR	NR	NR
			1 RH	NR	NR	NR
Hayashi et al. [19]	2011	2	NR	(270-575)	(40-330)	(7-14)
Tranchart et al. [20]	2011	2	1 LH	310	200	4
			1 RH	345	200	6

The patients with a single metastasis located in segments 2-6 are the ideal candidates for the simultaneous laparoscopic resection. Besides the standard location of trocars in laparoscopic colorectal surgery, 2 additional trocars are required, for a good access to perform the hepatic resection. Both resection specimens can be extracted through a single incision. In the case of lesions located in segments 7-8, or in the case a major hepatectomy was required, the simultaneous resection combined approach was aborted, and these patients were excluded from the study. However, certain authors with experience in laparoscopic liver surgery have proven that the laparoscopic major hepatectomy performed simultaneously with the laparoscopic colorectal resection is also feasible (18,20). The major hepatectomy specimen is extracted through a Pfannenstiel incision, which has been proven to have the lowest rate for the occurrence of an incisional hernia (21).

The theoretical arguments against simultaneous resection would be the combination between a clean operation and a contaminated one, and the deficiency on protein synthesis following the hepatic resection could lead to an increase of the infection risk and could compromise the healing of the anastomosis. Another hypothesis is related to the Pringle manoeuvre, when the resulted venous congestion could lead to intestinal edema. Nevertheless, the specialised literature based on 14 comparative studies reveals that the combined resection shows a lower morbidity rate (9). This led to the conclusion that simultaneous resection can be performed to patients selected by surgical teams specialised both in colorectal surgery, and in hepatobiliary surgery. The correct assessment of the patients, as well as their selection are essential for this type of complex surgery, and the multidisciplinary team must decide upon the optimal moment when the multimodal treatment must be applied.

As a result of the improvement of the surgical techniques in liver surgery, of the medical technical innovations (LigaSure™, Harmonic®, Enseal®) and of the systemic chemotherapy, the life expectancy of the patients with colorectal metastases increased. An initial laparoscopic approach results in a reduced formation of

peritoneal adhesions and it facilitates a repeated hepatic resection, which was proven in the case of patients with HCC that subsequently needed a liver transplant (22). The benefits of laparoscopy in this case can be seen in the improved oncologic results, in the improvement of the quality of life, the integrity of the abdominal wall and the cosmetic advantages.

CONCLUSION

The simultaneous resection of CRC and SLM continues to be a controversial issue. There are no randomized clinical studies comparing the simultaneous resection to the staged one. The recently communicated data of the prospective series are difficult to interpret because of the selective character of the patients. Our initial experience, correlated with the specialised literature, indicates that simultaneous resection of CRC and SLM is feasible and can be chosen for the patients selected, provided that it is performed by a team with adequate experience in this field.

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REZECTIA LAPAROSCOPICA SIMULTANA A CANCERULUI COLORECTAL SI A METASTAZELOR HEPATICE SINCRONE – STUDII INITIALE

REZUMAT

Rezecția simultană a cancerului colorectal (CCR) și a metastazelor hepatice sincrone (MHS) este un subiect de dezbatere legat de morbiditatea postoperatorie în comparație cu rezecția în etape. Scopul acestui studiu a fost analiza rezultatelor unei serii selectate de opt pacienți cu CRC și metastaze hepatice sincrone care au beneficiat de chirurgie laparoscopică combinată – colorectală și hepatică. Au fost evaluate prospectiv caracteristicile pacienților, caracteristicile tumorilor, variabilele operatorii cât și rezultatele postoperatorii. Tumora primară a fost localizată la nivelul colonului la șase pacienți și la nivel rectal la doi pacienți, iar metastazele hepatice sincrone au fost majoritar solitare. Prin abord laparoscopic la toți cei 8 pacienți,a fost realizatărezecția colorectala (R0) cât șimetastazectomii și rezecții hepatice atipice limitate. Nu a fost necesară conversia la chirurgia clasică. Extragerea piesei de rezecție-a făcut printr--o incizie pararectală sau suprapubiană transversală. Timpul operator mediu a fost de aproximativ 313 minute (interval 151-394 minute), cu o pierdere medie de sânge de 600 ml (interval 200-850 ml). Durata spitalizarii postoperatorii a variat între 6 și 14 de zile. Din această experiență inițială a unui singur centru, rezecția laparoscopică simultană colorectală și hepatică poate fi efectuată la pacienții selectați cu CCR și MHS, cu rezultate satisfacatoare pe termen scurt.

Cuvinte cheie: cancer colorectal, metastaze hepatice, chirurgie laparoscopică

PATIENT-PERCEIVED QUALITY OF MEDICAL SERVICES IN TIMIS COUNTY PUBLIC HEALTH HOSPITALS

BOGDAN CIPRIAN, BUCUR ADINA
"Victor Babes" University of Medicine and Pharmacy Timisoara

ABSTRACT

Introduction: The primary objective of hospital reform proposed by the Ministry of Health is to improve the quality of health services provided to insured persons and to increase access of all categories of population, including the disadvantaged. Currently, the concept of medical services clients's/users's response reaction is particularly emphasised in the public health system along with the idea of providing what he/she desires.

The aim of this paper is to assess patients' perceptions on the medical services and medical personnel professionalism in Timis County public health facilities.

Methodology: Three hundred seventy-three questionnaires were applied during 2013 year to randomly selected in-patients in Timis County hospitals. A set of questions were asked, out of which we selected for the purpose of this paper those highlighting the behaviour and professionalism of medical personnel, as well as aspects regarding medical care provided during continuous hospitalisation. Responses were collected in a database and processed by the means of SPSS 17.0 programme.

Results: Most patients were women and the best represented group was 41-50 years. Most patients showed a higher degree of dissatisfaction at questions about various aspects of medical care delivered. A higher proportion of patients declared themselves satisfied with the behaviour and professionalism of medical personnel. Regarding the general quality of medical services, 94.40% of patients are satisfied.

Conclusions: Although 90% of the interviewed patients address the same hospital and are satisfied with the quality of medical services in 94% of cases, they consider some aspects related to medical care and personnel should be improved.

INTRODUCTION

The primary objective of hospital reform proposed by the Ministry of Health is to improve the quality of health services provided to insured persons and to increase access of all categories of population, including the disadvantaged (1).

In the context of current reform, health facilities managers are more and more concerned with the improvement of the quality of services provided by the facilities they are managing (2,3).

Continuous quality improvement primarily aims to increase satisfaction of clients (both external and internal), as well as to decrease the cost of each delivered service (4).

Patient satisfaction mirrors the quality of health services.

Currently, the concept of medical services clients's/users's *response reaction* is particularly emphasised in the public health system along with the idea of providing what he/she desires (5,6).

Patient satisfaction is expressed as cognitive evaluation and emotional reaction to the elements of structure (human, material, financial resources and hospital environment), process (technical and interpersonal aspects), as well as to the results of medical services delivered to them (7).

Research in the field shows satisfaction is related to the perceived technical abilities, intelligence and qualification of medical personnel. However, patients mostly appreciate interpersonal communication skills of medical personnel medical (8).

First of all, patients' needs must be identified in order to enable medical personnel to address them using appropriate medical techniques and procedures (2,9).

Patients should be asked about their expectations from the physician. One frequently studied aspect of medical care is the extension and accuracy of physician-patient communication (2,4,10). Patient satisfaction is closely associated with accuracy of information provided and this could be an important indication of physician-patient communication quality (11).

The aim of this paper is to assess patients' perceptions on the medical services and medical personnel professionalism in Timis County public health facilities.

METHOD

In order to apply the questionnaires for patients, we have selected public hospitals in Timis County, except for children's and psychiatric ones. We have applied a non-probability sampling method, namely the fixed quota method, the monthly number of hospital admissions in each hospital being known. An average of 11.959 patients per month resulted from the total number of admissions in all the studied hospitals, from which we have calculated, by the means of SPSS 17.0 programme, a sample of 373 patients, with a +/- 3% error. The questionnaires were distributed among hospitals based on their proportion from the total number of admissions in all the studied hospitals. The questionnaire comprised preformed response questions, while

Received 24th of April 2014. Accepted 13th of May 2014. Address for correspondence: Adina Bucur, MD, PhD, Department of Functional Sciences, "Victor Babes" University of Medicine and Pharmacy Timisoara, Eftimie Murgu Sqare No. 2A, RO-300041, Timisoara, Romania, phone/fax: +40256490507, e-mail: adina.bucur@gmail.com

three were open-ended questions. Questionnaires were applied during 2013 year.

Table I. Distribution of patients in the sample that received questionnaires

Hospital	Patient percentage
County Clinical Emergency Hospital Timisoara	29.59%
City Clinical Emergency Hospital Timisoara	22.69%
Obstetrics-Gynaecology Clinical Hospital Timisoara	5.41%
Pneumo-phtisiology and Infectious Diseases Clinical Hospital Timisoara	4.66%
Cardiovascular Diseases Institute Timisoara	4.69%
Railway Hospital Timisoara	4.65%
Military Hospital Timisoara	5.55%
City Hospital Faget	3.81%
City Hospital Sannicolau Mare	5.41%
City Hospital Jimbolia	3.68%
City Hospital Deta	2.76%
City Hospital Lugoj	7.79%

RESULTS

The questionnaires were randomly distributed to in-patients in the Timis County public hospitals.

We have analysed the questions on patients' satisfaction regarding the quality of medical services provided to them during hospitalisation. The medical services quality is perceived by the patient as improvement of his/her health, which is conditional upon the correct assessment of patient's needs, so as the medical personnel to be able to address them using appropriate medical techniques and procedures. All these are based on the effective physician (medical personnel)-patient communication.

In the studied group, women represented the greatest proportion of admitted patients (Figure 1), with people between 41-50 years being the best represented age group, while two aggregated age groups (51-60 years and 61-70 years) were approximately equal with the 31-40 years age group.

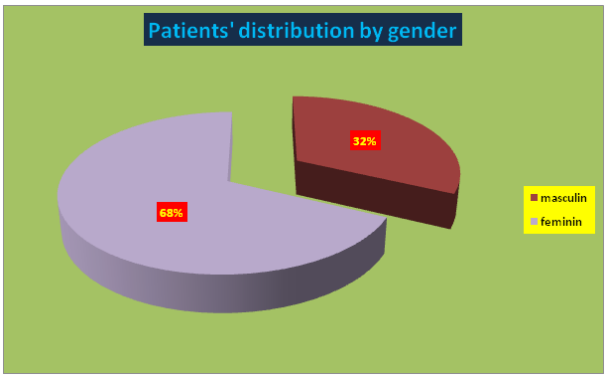

Fig. 1. Distribution of in-patients by gender

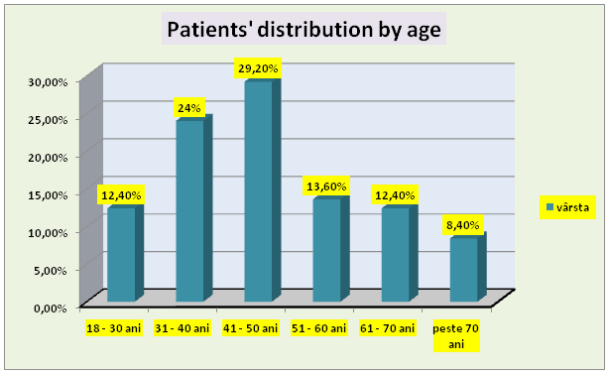

Fig. 2. Distribution by age groups

At the question regarding prior hospitalisation in the same health facility, 91% of patients chose the same hospital, which is most probably due to hospital proximity and not to medical services. Furthermore, when only one department for a certain medical specialty is available in the county, the patient will be re-admitted in the same department for the same pathology.

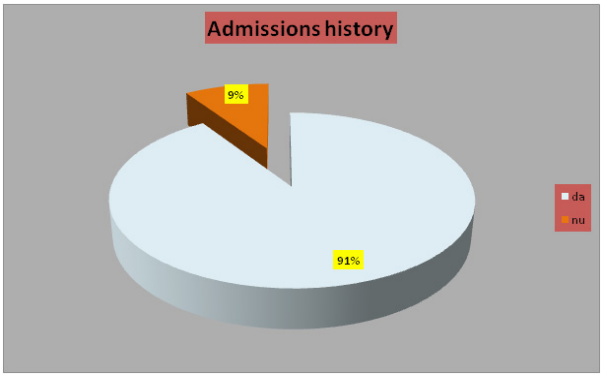

Fig. 3. History of admissions in the same health facility

In defence of the previously mentioned reasons for choosing the same hospital, only 42% would choose the same health facility if free option, with no financial or service uniqueness constraints would be available.

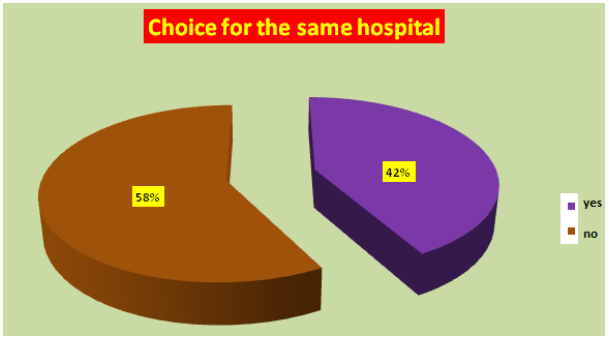

Fig.4. Distribution of patients according to the choice for the same health facility

Regarding the admission method, only 12% have been admitted through the emergency department.

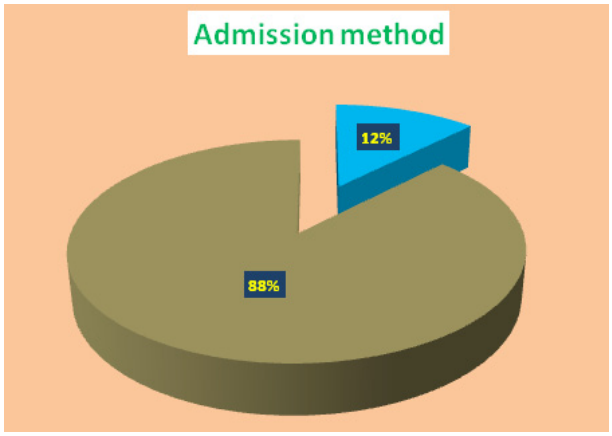

Fig. 5. Distribution of patients according to admission method

Most of the patients (77.20%) were waiting between one and two hours to be examined for hospitalisation.

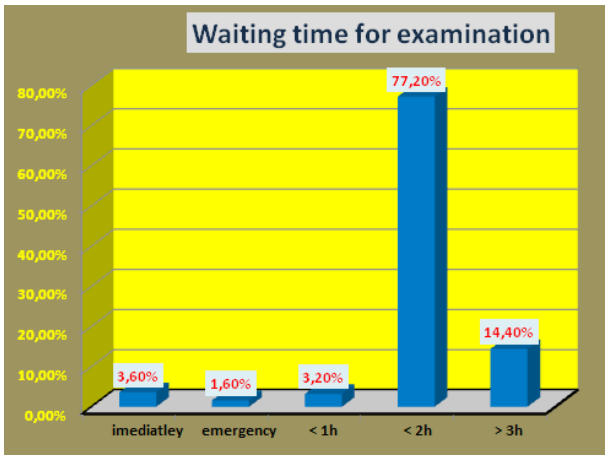

Fig. 6. Distribution of answers regarding waiting time for examination

Out of the total number of patients, 89.20% assert medical tests were performed for them and only 10.80% came with medical tests already done in the outpatient setting; patients proved to be satisfied in this respect.

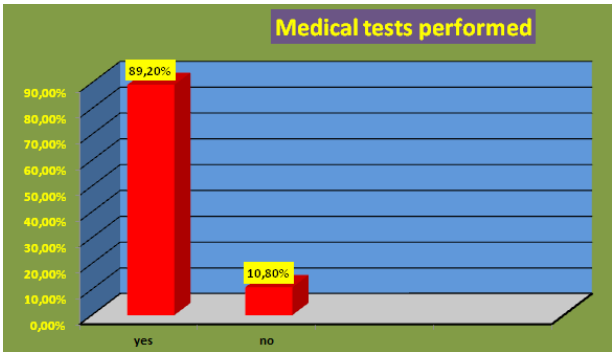

Fig. 7. Distribution of answers according to medical tests performed

On question whether they were accompanied by medical personnel to the various procedures recommended during hospitalisation, the proportions were almost equal for accompanied versus not accompanied patients. Among patients, 23.60% were accompanied by relatives.

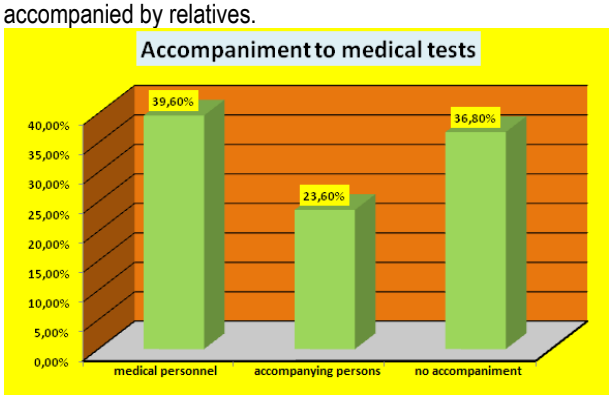

Fig. 8. Distribution of answers regarding the method of accompaniment to various medical procedures

Treatment is another important component of health services. On the question whether patients have purchased or not medicines for their treatment in the hospital, only 4.4% said their treatment was exclusively received from the hospital, 28.80% have both received and bought medicines, but the vast majority (66.80%) have bought their medicines.

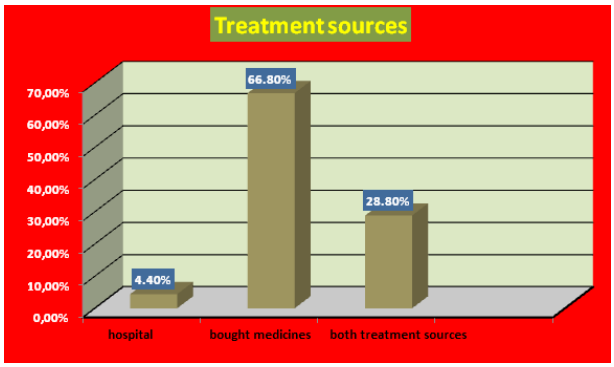

Fig. 9. Distribution of answers regarding treatment source

In respect of the information received on various requests, patients are fully dissatisfied (91.20%).

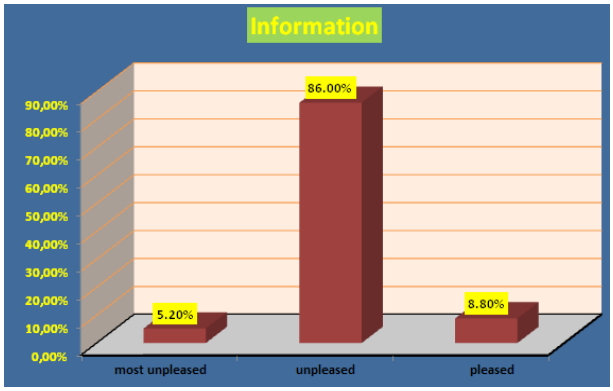

Fig. 10. Distribution of answers regarding information received on various requests

Interaction with medical personnel and their professionalism is also a component of the medical services quality. The attending physician's attitude during his/her interaction with the patient was considered good and very good (94.40%), even though approximately 50% were satisfied with the time devoted to consultation (Figure 12).

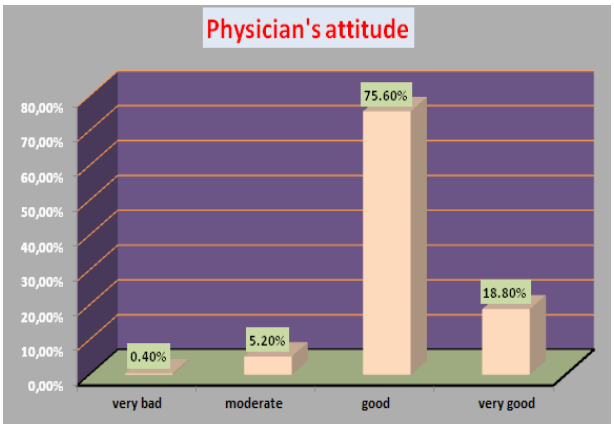

Fig. 11. Distribution of answers regarding physician's attitude

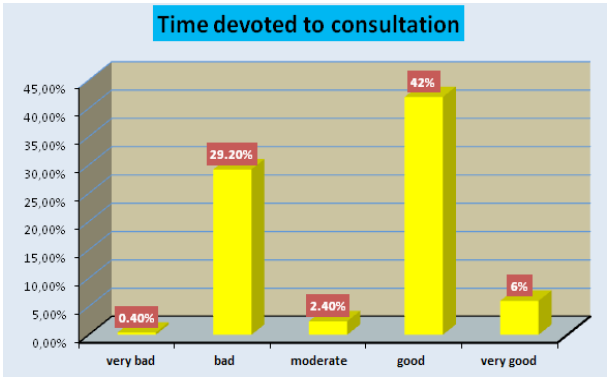

Fig. 12. Distribution of answers regarding the time devoted to consultation

Physician's professionalism is difficult to judge. However, 90% consider the attending physician was well qualified, although only 64% consider they have received enough information regarding their disease.

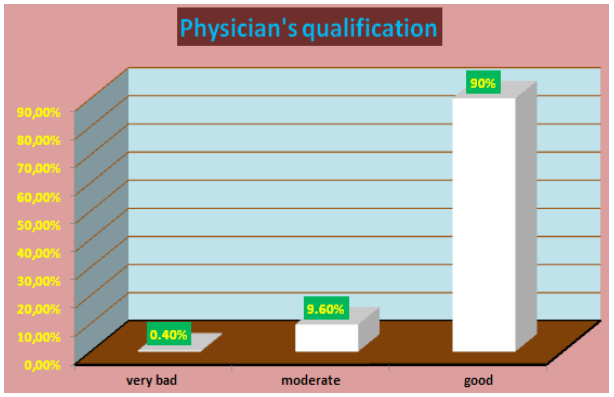

Fig. 13. Distribution of answers regarding physician's qualification

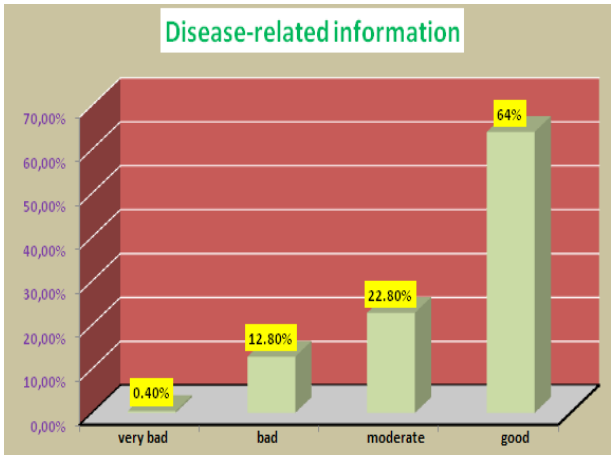

Fig. 14. Distribution of answers regarding disease-related information

Behaviour of nurses was judged as well, approximately 50% of patients being satisfied by their behaviour, 30% being dissatisfied with their professionalism and 70% judging it as appropriate.

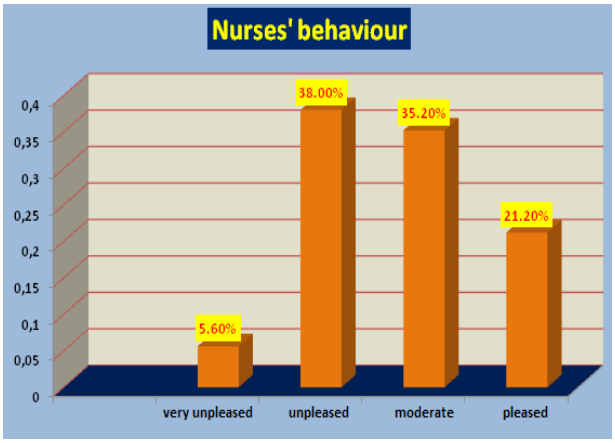

Fig. 15. Distribution of answers regarding nurses' behaviour

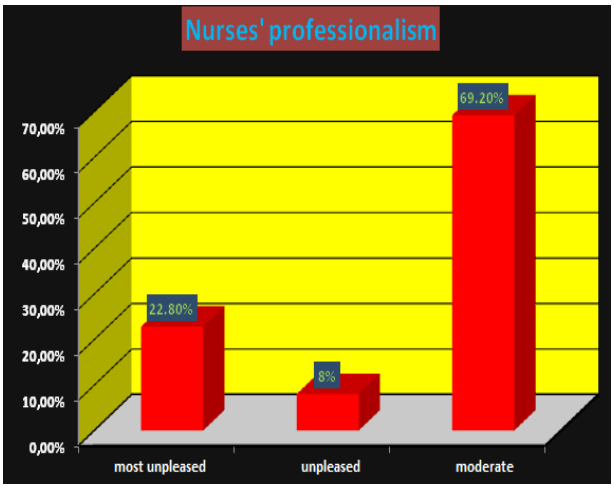

Fig. 16. Distribution of answers regarding nurses' professionalism

Regarding the general quality of medical services, 94.40% of patients are satisfied.

Fig. 17. Distribution of answers regarding quality of medical services

DISCUSSIONS

The public opinion barometer conducted in 2006 among Romanian citizens shows that people with a less or more severe health problem choose primarily the family/specialist physician's office (60.47%) and only 17.6% address emergency services (emergency room - UPU) (12). This is in accordance with our study, where admissions through emergency services account for 12% of total admissions within the study group. In our study, 90% of people would choose the same hospital, which is in agreement with data in the literature showing three quarters of the individuals in the general population hospitalised during 2006 year would choose the same hospital in the event of a re-admission.

During hospitalisation, 90% of the questioned patients in the general population received explanations about their diagnosis, 85% about the procedures and interventions they underwent or about disease prognosis and evolution (12). Only half of the hospitalised individuals received information regarding their rights to refuse a medical intervention, to receive an alternate medical opinion or about alternatives to the proposed procedures (12). In our study, 86% of the interviewed people are dissatisfied with the information received to various requests, while 86.8% are satisfied with information they received about their disease compared to general population.

Half of the in-patients assert there were certain negative aspects during hospitalisation related to services delivered, but only one in ten individuals has complained about this to some other person (family member, nurse or physician) (12).

One of five persons considers medical personnel attention and behaviour is the most important aspect that should be changed in hospitals (12). Fifteen percent of the respondents consider endowment with medical equipments and unofficial payments are the most important aspects that need to be changed (12).

Medical personnel professionalism is an important aspect asserted by 8.5% of patients (12). These data are in support of our study, in which the interviewed patients are satisfied with the physician's attitude (94%), although 50% are satisfied with the time devoted to consultation and 90% with the physician's qualification. A great part of patients are dissatisfied with nurses' behaviour (43.6%), even though 70% are satisfied with the medical care they provide.

The quality of medical services provided by Romanian hospitals is judged good and very good by approximately one third of respondents (12). Similarly, one third of respondents judge the quality of medical services as average and one quarter consider they are poor or very poor (12). In our study, 50.8% of patients consider the quality of medical services is average and only 43.6% judge it as good and very good.

CONCLUSIONS

Although 90% of the interviewed patients address the same hospital services and 94% of them are satisfied with the quality of medical services, there are also aspects that should be improved regarding the medical services and the medical personnel: better communication with the patient of both physicians and nurses, enough time devoted to patient and better qualification of personnel.

Regarding medical services, patients' discontent is mirrored by the fact that they must purchase their medicines for in-hospital treatment, which should be incumbent to the health facility.

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Barometrul de opinie privind Serviciile de sănătate realizat în rândul populaţiei din România, Centrul pentru Politici şi Servicii de Sănătate Bucureşti, decembrie 2006

PERCEPTIA PACIENTILOR REFERITOARE LA CALITATEA SERVICIILOR MEDICALE IN SPITALELE JUDETULUI TIMIS

REZUMAT

Introducere: Obiectivul principal al reformei spitalelor propusa de Ministerul Sanatatii este acela de a imbunatati calitatea serviciilor medicale oferite persoanelor asigurate si cresterea accesului pentru toate categoriile populationale, inclusiv persoanele dezavantajate. In prezent, este promovat mai ales conceptul de servicii medicale bazat pe reactia de raspuns a clientului/utilizatorului in sistemul de sanatate publica, impreuna cu idea de a asigura ceea ce doresc acesti utilizatori

Scopul acestui studiu este de a testa perceptia pacientilor asupra serviciilor medicale si a profesionalismului personalului medical din institutiile de sanatate publica ale judetului Timis.

Metodologie: Au fost aplicate 373 de chestionare in decursul anului 2013 pacientilor spitalizati in spitalele judetului Timis. Au fost formulate o serie de intrebari, din care am ales pentru scopul acestui studiu pe acelea care subliniau comportamentul si profesionalismul personalului medical, precum si aspectele legate de asistenta medicala oferita in timpul spitalizarii. Raspunsurile au fost introduse intr-o baza de date si procesate cu ajutorul programului SPSS 17.0.

Rezultate: Majoritatea pacientilor au fost de sex feminin si cel mai bine reprezentat grup a fost cel cu varste cuprinse intre 41-50 ani. Majoritatea pacientilor au prezentat un grad crescut de insatisfactie la intrebarile legate de diferite aspecte ale serviciilor medicale oferite. O proportie crescuta de pacienti s-au declarat satisfacuti de comportamentul si profesionalismul personalului medical. Referitor la calitatea generala a serviciilor medicale, 94.40% dintre pacienti au fost satisfacuti.

Concluzii: Cu toate ca 90% dintre pacientii intervievati s-au adresat aceluiasi spital si au fost satisfacuti de calitatea serviciilor medicale in 94% din cazuri, acestia considera ca anumite aspecte legate de asistenta medicala si personalul medical ar trebui imbunatatite.

LONG-TERM EFFECTS OF SIMVASTATIN THERAPY ON CHRONIC INFLAMMATORY AND AUTOIMMUNE DISEASES

ELENA CIURARIU[1], VICTOR DUMITRAŞCU[2,3]

[1]Department of Functional Sciences," Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania
[2]Pharmacology Department," Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania
[3]Timisoara County Emergency Clinical Hospital, Romania

ABSTRACT

Objective. To examine the immunosuppressive effects mediated by long-term therapy with simvastatin in a trial of patients with chronic inflammatory and autoimmune diseases..

Patients and Methods. Fifty-five eligible patients participated in this randomized, open, retrospective and comparative survey (study group). The patients, ranging in age from 16 to 76 years, redeemed a prescription for simvastatin for impaired lipidic profile. Inclusion criteria: patients were classified in three subgroups, accordingly with their certified chronic inflammatory and autoimmune disease: rheumatoid arthritis (RA) (n = 23), lupus erythematosus (LES) (n = 22), and respectively sclerosis multiple (SM) (n = 10). Simvastatin was administered during a 6-month period in a daily evening dose of 40 mg, as an adjunct to existing disease-modifying antirheumatic drug therapy.

Patients were followed up over 6 months. Clinical disease activity variables and acute-phase reagents were analyzed comparatively.

Results. Three major diseases have been revealed in the study group, respectively: 67 % cases of rheumatoid arthritis (RA), 22% cases of lupus erythematosus (LES) and 11% of sclerosis multiple (SM). After simvastatin treatment, pain relief mobilization was significantly improved in 53% of cases of RA, 27% cases with LES and 20 % cases of SM. 63 % of the patients with RA improved their physical activity. C-Reactive Protein (CRP) and erythrocyte sedimentation rate declined by 67% and 35%, respectively after 6-month simvastatin therapy and interleukin -6 (IL-6) decreased from 107.22 pg/mL to 18.16 pg/mL.

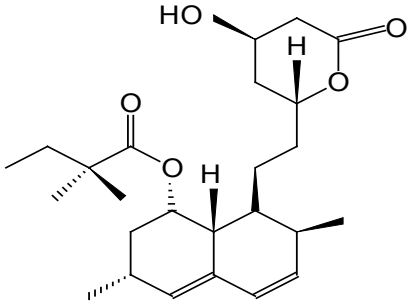
Conclusion. In addition to the beneficial effects of cholesterol reduction, immune modulation in chronic inflammatory and autoimmune diseases may contribute to the cardio protective effect of simvastatin.

Key words: simvastatin, immunomodulatory role, chronic inflammatory and autoimmune diseases

INTRODUCTION

Simvastatin belongs to the most widely class of drugs used as lipid-lowering agents. Based on their pleiotropic spectrum of activity, several studies revealed complex immunological pathways modulated by statin therapy (1,7).

Simvastatin acts as a 3-hydroxy-3methylglutamyl-coenzyme A (HMG-CoA) which catalyses the conversion of HMG-CoA to mavelonic acid (Figure 1):



Simvastatin

Fig. 1. Chemical structure of simvastatin

Published studies revealed immunological effects that indicate the impact of statin therapy on immune inflammatory responses (3). Simvastatin decreases chemotaxis and lipopolysaccharide mediated release of tumour necrosis factor-α (TNT- α).

Previous researchers have pointed out the important role of simvastatin on bone remodeling by the finding that nitrogen-containing bisphosphonates exert their cytotoxic effects on osteoclasts by interfering with the mevalonate pathway (5,9). Long-term simvastatin treatment exerts a dual beneficial pharmacological and clinical effect on the lipidic profile and bone mineral density, demonstrating positive consequences in clinical practice, as we revealed in a 2-years research (2).

Systematically review of the literature data point out the non-lipidic mechanisms – called "pleiotropic", which have as a result the lowering of inflammation by simvastatin therapy. Most of these studies focus on the rheumathoid arthritis (RA), because this disease is associated with a higher risk of cardiovascular disease (12).

The aim of the present study was to retrospectively evaluate the immunosuppressive effects mediated by long-term therapy with simvastatin in a trial of patients with chronic inflammatory and autoimmune diseases.

MATERIALS AND METHODS

Fifty-five eligible patients participated in this randomized, open, retrospective and comparative survey (study group). The patients, ranging in age from 16 to 76 years, redeemed a prescription for simvastatin for impaired lipidic profile.

Inclusion criteria: patients were classified in three subgroups, accordingly with their certified chronic inflammatory and autoimmune disease: rheumatoid arthritis (RA) (n = 23), lupus erythematosus (LES) (n = 22), and respectively sclerosis multiple

Received 25th of April 2014. Accepted 15th of May 2014. Address for correspondence: Elena Ciurariu, MD, PhD student, Department of Functional Sciences, "Victor Babes" University of Medicine and Pharmacy, *2 Eftimie Murgu Square*, 300041 *Timisoara,* Romania, phone: +40 723081655; e-mail: ciurariu.elena@gmail.com

(SM) (n = 10).

Exclusion criteria: patients with multiple co-morbidities, drug abuse, intolerance to simvastatin or previous statin therapy within the last 6 month.

This study was performed between February 2013- July 2013, in accordance with the ethical standards of the responsible institutional committee on human experimentation and with the declaration of Helsinki, as revised in 2000. All the participants gave informed consent and were recruited from the patient lists of family healthcare providers from Timisoara, Romania.

Simvastatin was administered during a 6-month period in a daily evening dose of 40 mg, as an adjunct to existing disease-modifying antirheumatic drug therapy.

Patients were followed up over 6 months. Clinical disease activity variables and acute-phase reagents were analyzed comparatively.

Sera from all the 55 patients from the study group were analyzed for interleukin-6 (IL-6) by enzyme-linked immunosorbent assay (ELISA) using commercially available kits; baseline plasma levels were compared with those obtained after 6 month of simvastatin therapy.

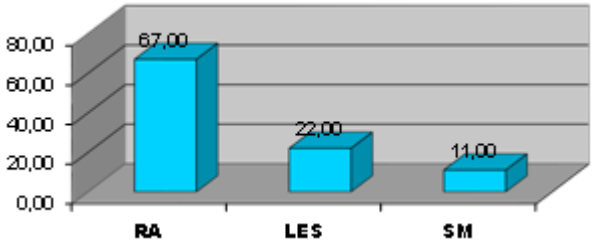
All statistical analyses were performed using the SPSS software package (version 6.0 for Windows, SPSS Inc, Chicago, IL.).

RESULTS AND DISCUSSIONS

Of the patients from the study group, 5 (9.09 %) were aged between 16 and 30 years, 9 (16.36 %) between 31 and 40 years, 12 (21.82 %) between 41 and 50 years, 22 (40 %) between 51 and 60 years , 7 (12.73%), between 61 and 74 years. The group consisted of 55 patients, 33 females (60 %) and 23 male patients (40 %).

The study revealed maximum incidence of chronic and autoimmune disorders which corresponds to the period 51-60 years, female sex predominance.

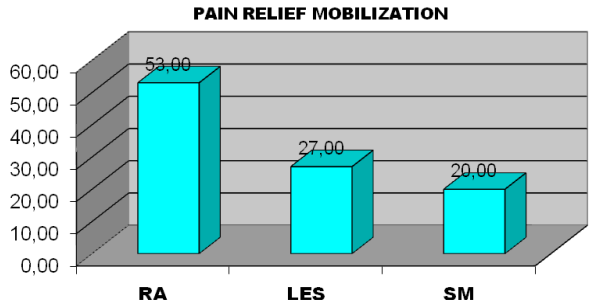
From the viewpoint of clinical diagnosis, three major disease have been revealed in the study group, respectively: 67 % cases of rheumatoid arthritis (RA) , including bilateral form prevailed in 53% of cases compared with the unilateral - 18 % of cases, 22% cases of lupus erythematosus (LES) and 11% of sclerosis multiple (SM) (Figure 2).



Legend: RA = Rheumatoid Arthritis; LES = Lupus Erythematosus; SM = Sclerosis Multiple

Fig.2. Patient distribution in the study group according to the type of autoimmune disease

In terms of clinical simvastatin treatment administered for six month, pain caused by joint mobilization was observed in all cases studied initially (100 %); after simvastatin treatment, pain relief mobilization was significantly improved in 53% of cases of rheumatoid arthritis (RA), 27% cases with lupus erythematosus (LES) and 20 % cases of sclerosis multiple (SM) (Figure 3).



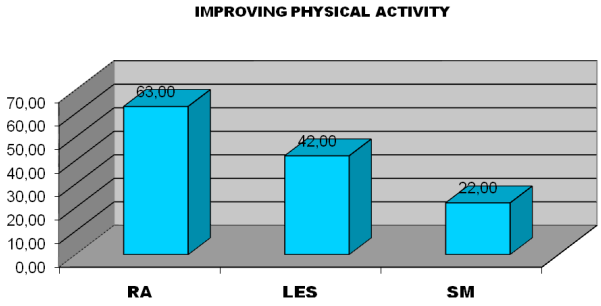
Legend: RA = Rheumatoid Arthritis; LES = Lupus Erythematosus; SM = Sclerosis Multiple

Fig. 3. Pain relief mobilization according to the type of autoimmune disease

Functional impotence associated with the degree of limitation of physical activity of the patient under investigation was significantly improved after 6 month treatment with simvastatin, and the best results were the following (figure 4):

- 63 % of the patients with rheumatoid arthritis (RA) improved their physical activity;
- 42 % of the patients with lupus erythematosus (LES) and
- 22 % of the patients with sclerosis multiple (SM).

These results highlight the immunomodulatory role of simvastatin in rheumatoid arthritis (RA), which acts by decreasing the inflammatory synovitis, articular destruction, and accelerated atherogenesis, characteristic for all the chronic inflammatory and autoimmune diseases (11).



Legend: RA = Rheumatoid Arthritis; LES = Lupus Erythematosus; SM = Sclerosis Multiple

Fig. 4. Improving physical activity according to the type of autoimmune disease

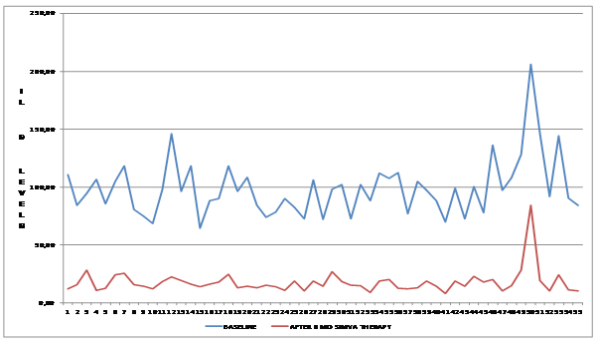
All these effects contribute to improving patient's lives, translated by reducing pain at rest and on mobilization, increased general joint mobility, improved walking tolerance and decrease the patient's debilitating physical dependence.

Compared to baseline, C-Reactive Protein (CRP) and erythrocyte sedimentation rate declined by 67% and 35%, respectively after 6-month simvastatin therapy. In the study group, CRP decreased from 21 to 8.40 mg/dl;a significant drop in plasma CRP level was achieved after the six month of simvastatin treatment.

A special marker of inflammation is Interleukin-6 (IL-6), which had as baseline a mean serum concentration of 107.22 pg/mL (range 68.52 - 146.40 pg/mL). From these, 22 cases (40%) recorded levels higher than100 pg/mL, ten times the upper limit of normal. Cases with lupus erythematosus (LES) recorded these high values of IL-6 at baseline, as described in the literature (10). After 6 month of simvastatin therapy, the IL-6 levels decreased

significantly, achieving a mean concentration of 18.16 pg/mL (range 8.78 - 84.12 pg/mL).

Interleukin-6 (IL-6) variations are revealed in Figure 5:



Legend: IL-6 = Interleukin-6

Fig.4. Comparison of Interleukin-6 levels at baseline and after 6 month of simvastatin therapy in patients with autoimmune disease

Extensive experimental studies and more recently many clinical trials have strongly suggested statins to possess an important role in chronic inflammatory and autoimmune diseases, mainly mediated by their anti-inflammatory and immunumodulatory properties (4,8).

Our results are in accordance with those of previous researches, who revealed that simvastatin reduces proinflammatory cytokine production *in vitro* by T cell contact-activated macrophages in peripheral blood and synovial fluid (6).

The greatest improvements have been revealed in patients with more active autoimmune diseases.

The presented study has some limitations: One limitation is the small sample size and the broad.confidence interval. The precise mechanisms of simvastatin action on autoimmune diseases will be clarified by further studies.

CONCLUSIONS

In addition to the beneficial effects of cholesterol reduction, immune modulation in chronic inflammatory and autoimmune diseases may contribute to the cardio protective effect of simvastatin.

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EFECTELE PE TERMEN LUNG ALE TRATAMENTULUI CU SIMVASTATIN IN AFECTIUNILE INFLAMATORII CRONICE SI AUTOIMUNE

REZUMAT

Obiective. Investigarea efectelor imunosupresoare mediate de terapia pe termen lung cu simvastatina, pe un lot de pacienti cu afectiuni inflamatorii cornice si autoimmune.

Pacienti si Metode. Au participat 55 de pacienti eligibili la acest studiu randomizat, deschis, retrospectiv si comparativ (grup de studiu). Pacientii, cu varste cuprinse intre 16 si 76 de ani au primit prescriptii medicale pentru simvastatin in cazuri de alterare a profilului lipidic. Criterii de includere: pacientii au fost clasificati in trei subgrupuri, in functie de diagnosticul pozitiv pentru afectiuni inflamatorii cornice si autoimune: artrita reumatoida (RA) (n = 23), lupus eritematos (LES) (n = 22), si respectiv scleroza multipla (SM) (n = 10). Simvastatina a fost administrata pe o perioada de 6 luni in doza zilnica de 40 mg, seara, ca tratament asociat terapiei anti-reumatice existente. Pacientii au fost urmariti pe o perioada de 6 luni. Au fost analizate comparativ variabilele de activitate ale bolii si parametrii de faza acuta.

Rezultate. Au fost evidentiate trei afectiuni majore in grupul de studiu: 67 % cazuri de artrita reumatoida (RA), 22% de cazuri de lupus eritematos (LES) si 11% de scleroza multipla (SM). Dupa tratamentul cu simvastatin, mobilizarea ca urmare a reducerii durerii a fost imbunatatita semnificativ in 53% din cazurile de RA, 27% din cazurile de LES si 20 % din cazurile de SM. 63% dintre pacientii cu RA au prezentat o imbunatatire a activitatii fizice. Preoteina C reactiva (CRP) si viteza de sedimentare a eritrocitelor au prezentat o scadere cu 67% si respectiv cu 35%, dupa 6 luni de terapie cu simvastatina, iar interleukina 6 (IL-6) a prezentat o scadere de la 107.22 pg/mL la 18.16 pg/mL.

Concluzie. Aditional efectelor benefice asupra reducerii valorii colesterolului, modularea imuna in afectiunile cornice inflamatorii si autoimun ar putea avea o contributie la efectele cardioprotectoare ale simvastatinei.

Cuvinte cheie: simvastatina, rol imunomodelator, boli cornice inflamatorii şi autoimune.

PROSPECTIVE STUDY OF LAPAROSCOPIC GREAT CURVATURE PLICATION – EFFECTS ON WEIGHT LOSS AND HUNGER HORMONE LEVELS

DOBRESCU AMADEUS, VERDEŞ GABRIEL, TARȚA CRISTI, BREBU DAN, STOICA LAURIAN, LAZĂR CAIUS, DUȚĂ CIPRIAN

Second Surgery Clinic of Timisoara County Hospital,"Victor Babeș" University of Medicine and Pharmacy Timișoara

ABSTRACT

Introduction: Laparoscopic greater curvature plication (LGCP) introduces no implantable device, requires no gastric or intestinal resection or intestinal bypass, and one of the most important advantages is that this technique is potentially reversible; also it can be followed by more extensive procedures. Aim of our study is to evaluate the excess weight loss (EWL) and the ghrelin levels changes in a series of obese patients that underwent LGCP.

Methods: This is a prospective non-randomized study and includes all the patients operated with LGCP during 2012 in Second Surgical Unit of the Timisoara County Hospital.Data were collected from the patients' files, questionnaires and from specified follow-up visits at 1, 3, 6 and 12 months. All the patients fulfilled the follow up at 12 months at the moment of this writing.Main intervention was considered the LGCP. Main outcome was excess body weight loss (EWL), secondary outcomes were ghrelin levels, modified hunger and satiety sensations, weight regain, surgical complications, readmissions, intra-operative data – operating time, blood loss.

Results:14 patients were included in the study – they underwent LGCP, 12 females, 2 males. All the procedures were performed entirely laparoscopic. EWL was at 1 month - 25.7%, 3 months - 34.9%, 6 months - 44.9%, 12 months - 57.6%. Before surgery the median level of ghrelin was 283.05 pg/ml, after surgery the level dropped at 160.60 pg/ml at 1 month and to 65.40 pg/ml at 3 months. Comorbidities resolve in 4 patients out of 8 patients. There were two minor complications – nausea and vomiting that prolonged the hospital stay and needed conservative treatment. Two patients had less weight loss due to diet and lack of motivation.

Conclusion: LGCP can be a successful bariatric procedure performed by a trained bariatric surgeon on a population of selected patients with strong motivation. EWL is satisfactory; complications are rare and mild and usually need only conservative treatment.

Key words: ghrelin, laparoscopic placation, weight loss

INTRODUCTION

Of all treatments for obesity, metabolic/bariatric surgery has shown the greatest sustained effectiveness in achieving weight loss and comorbidity improvement, with social reinsertion of the subjects. The evolution of our society with the food not being a concern for ordinary people results in an increased trend in prevalence of morbid obesity around the world (1,2). Same thing happened in Romania since the introduction of the fast-food culture. Influence of diet and exercise in morbid obese patients is about 10% in long term period; thus, in case of life-style modification failure, bariatric surgery could be considered (3).

Traditionally, the primary mechanisms through which bariatric surgery achieves its outcomes are believed to be the mechanical restriction of food intake, reduction in the absorption of ingested foods, or a combination of both (4). More or less all the bariatric procedure lead to a partial resection of the stomach, opening of the stomach or of the small bowel, either leave implants consisting in different devices. The very few prior preclinical and clinical studies of laparoscopic greater curvature plication (LGCP) — the newest operation in metabolic surgery — suggest the potential for LGCP to provide safe, significant weight loss and improvement of metabolic parameters similar to that shown by currently accepted restrictive metabolic procedures. LGCP introduces no implantable device, requires no gastric or intestinal resection or intestinal bypass, and one of the most important advantages is that this technique is potentially reversible; also it can be followed by more extensive procedures (5).

Nevertheless the technique is the less expansive bariatric procedure, a major advantage in a developing country with more and more obese and no proportional health financing.

Since introduction of the technique by Talebpour in 2007 (6), there are few studies evaluating the technique and the outcomes, the largest one published by the same author in 2012 consisting in about 800 patients (7).

Our study is a prospective series including all the patients operated in our hospital in 2012 with a LGCP and followed for a year, we also determined the hunger hormone ghrelin at 0, 1 and 3 months post-operatively and applied questionnaires about hunger and satiety feelings before and after the procedure.For our knowledge this is the first series published from our country.

METHODS

This is a prospective non-randomized study and includes all the patients operated with LGCP during 2012 in Second Surgical

Received 15th of June 2014. Accepted 20th of July 2014. Address for correspondence: Amadeus Dobrescu, MD, PhD Student, Department of Surgery, University of Medicine and Pharmacy "Victor Babeș" Timișoara, Iosif Bulbuca Street, No. 10, Timișoara, România; phone: +40740015355, e-mail address: amadeusdobrescu@gmail.com

Unit of the Timisoara County Hospital.Data were collected from the patients' files, questionnaires and from specified follow-up visits at 1, 3, 6 and 12 months. All the patients fulfilled the follow up at 12 months at the moment of this writing.

Main intervention was considered the LGCP. Main outcome was excess body weight loss (EWL), secondary outcomes were ghrelin levels, modified hunger and satiety sensations, weight regain, surgical complications, readmissions, intra-operative data – operating time, blood loss.

Surgical technique

The patient was placed in 30° reverse Trendelenburg position, with the operating surgeon standing between patient legs. Pneumoperitoneum was created inserting the Veress' needle at the Palmer's point, left mid clavicular line below the last rib either on the linea alba at two thirds below xyphoid or using the open Hasson technique for the insertion of the first trocar if previous major abdominal surgery was encountered.

The patient was placed in 30° reverse Trendelenburg position, with the operating surgeon standing between patient legs. Dissection started at the greater curvature of the stomach from the middle of the antrum and continued to the left diaphragmatic crus, and downwards to the level of the pyllor. Communicating vessels were ligated by LigaSure (LigaSureTMCovidien, USA), Ultracision (Harmonic® Ultrasonic, Ethicon, USA) or clips. Continuous suturing from 2 cm below the cardia through the antrum, 4 cm distance above the pyllorus, was performed in this stage, making one and then two layers of plicated stomach from the anterior wall of the stomach to the posterior wall. For suturing 2-0 prolene (2-0 PROLENE™ Polypropylene Suture, Ethicon, USA) was used, and the bulk of each stitch was 1 cm with a 1-cm interval. For gastric tube calibration we used a 36 Fr tube. Sutures were extramucosal, preventing absorption by gastric acid (8).

Drainage was used for 24 hours in order to see if there was any bleeding.

Post-operative we administrated to all the patients proton pump inhibitors 2*40 mg/day, anti-inflammatory and anti-nausea/vomiting drugs. Low molecular heparins were administrated for up to three weeks to prevent deep vein thrombosis and superior mesenteric vein thrombosis.

Postoperative diet was resumed 24 hours after surgery and consisted only in fluids up to the 9th POD. Next three weeks all the food will be semi-liquid.

Measurements were performed for all patients at the five study time points (baseline, 1, 3, 6 and 12 months post-op), as per protocol. Body weight was measured to the nearest 0.5 kg and height to the nearest 1 cm. BMI was calculated as body weight in kilograms divided by the square of the height in meters. Percent excess weight loss (%EWL) was calculated according to the following equations:

%EWL = [(preoperative weight − follow-up weight)/preoperative weight-ideal body weight] x100

Questionnaires were applied at 0, 1, 3, 6 and 12 months regarding hunger and satiety sensations. Patients were required to evaluate on a scale from 1 to 10 these sensations. Weak hunger sensation was noted as 1, strong sensation as 10. Rapid satiety was noted as 1, whereas 10 counts for slow satiety and the need for large meals to reach satiety.

RESULTS

14 patients were included in the study – they underwent LGCP. All the procedures were performed entirely laparoscopic. In the Table I we have the demographics and the peri-operative data of the patients. 8 patients had comorbidities: hypertension, diabetes mellitus type II, dyslipidemia, osteo-articular disease and obstructive sleep apnea.

Table I. Demographics and the peri-operative data

Patients	n=14
Sex ratio F:M	12:2
Age (years) *	43
Other comorbidities	57.14%
OR time(minutes)*	51 (40 - 102)
Hospital stay(days)*	3 (2 - 10)

*median value

There was no intra-operative blood loss. There was only one readmission on the 3rd post-operative day (POD) due to incontrollable vomiting and nausea, followed by dehydration. The treatment was conservative with intra-venous anti-inflammatory drugs; proton pump inhibitors; parenteral fluids and nutrients. The patient was discharge 8 days later with resumed oral food intake and with no nausea or vomiting. Proton pump inhibitors, anti-inflammatory and anti-nauseadrugs were prescribed per mouth. Another patient had vomiting and nausea during hospital stay for 10 days – same treatment was prescribed.

The results, in terms of BMI and EWL, of the procedure are summarized in Table II. There were 2 patients lost to follow-up, one at 6 month and another one at 12 months. Two patients had less EWL, a male and a female, after re-screening they prove to be sweet eaters.

Table II. BMI and EWL results

	0	1 month	3 months	6 months	12 months
BMI (kg/m^2)	38.6	33.9	31.8	30.2	28.6
EWL %	-	25.7	34.9	44.9	57.6

BMI – body mass index; EWL – excess weight loss; average value was used

There were no long-term complications. All the patients answered the questionnaires about hunger and satiety sensation and the results are shownin Figure 1.

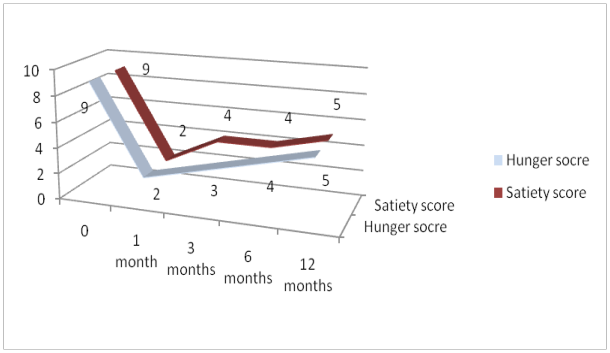

Fig. 1. Hunger and satiety sensation score

Ten patients had ghrelin blood levels determined at 0, 1 and 3 months. Before surgery the median level of ghrelin was 283.05 pg/ml, after surgery the level dropped at 160.60 pg/ml at 1 month and to 65.40 pg/ml at 3 months.

Comorbidities diminished at the end of the follow-up, there were present in 4 out of 12 patients. Number of deaths were recorded.

DISCUSSIONS

There few advantages of the LGCP – potentially reversal procedure, lower cost, no resection, no foreign body inside the abdomen and a low rate of complications. One of the disadvantages is the lack of a randomized trial.

No need for a reversal of LGCP in our study occurred, Talebpour et al reported that reversal is possible in the first six weeks after surgery, later on dense fibrotic adhesions are formed between folds (7). Other authors also reveal that LGCP could be easily reversed in an early postoperative period, before formation of dense adhesions between the layers of the plication (9).

There were no major complications recorded in our 14 patients' series. The rate of complication was 14.28%, consisting only in minor complications – nausea and vomiting, percentage that is mentioned in the literature, our study scarce number of patients could be a reason for the relative high percentage of minor complications, Talebpour *et al* reported 16 % complications. First the LGCP was performed by a single row of sutures, the remaining volume of the gastric tube was difficult to assessed, then two rows were used and the gastric volume got smaller. In a detailed description of the technique Talebpour described the difference between the two methods the average anatomic volume of stomach in the operating room was 100 and 50 cc in one- and tow-row plication respectively; the functional volume of stomach in one- and two-row LGP respectively was about 25 and 15cc at first, 50 and 25cc after 2 weeks, 75 and 45cc after6 months, 100 and 60cc after 1 year and 250 and 150cc after 4 years (7). Looking at the relative small volume of the remaining gastric tube the post-operative gastric wall edema can easily temporary induce nausea and vomiting. Same author described the vomiting as common encounter two days after surgery (7).

EWL was recorded at 0, 1, 3, 6 and 12 months. Largest amount of EWL was at the patients with BMI lower than 40 kg/m^2, most likely due to more rapid social reinsertion, possibility to practice physical exercices. Talebpour recommended special filters for the candidates willing to have performed LGCP, strong motivation and discipline can play a mjor role in weight loss and is the key factor in maintaining the EWL. The longer the follow-up the worse are the EWL (10). In our sudy the results are improved comparing to other series on short term – at one month 25.7% versus 20% in Talebpour series, latter on the results are surpassed by other as most likely the lack of obese support group determined patients to fail the program they were enrolled. In two patients the EWL was low, after reviewing the interviews we found out that they were sweet eater, a relative contraindication of the restrictive method.

The 57.6% EWL a year after surgery qualified the LGCP as a good choice of bariatric surgery, main drawback being the selection of the patients; method has its limit when we cross the border of 40 kg/m^2.

Comorbidities rates dropped after EWL consecutive to LGCP. In our study there is a direct relation between EWL and comorbidities resolution. At 12 months follow-up EWL was 57.6%, and the comorbidities rate was lower with 40%.

Hunger sensation of the obese persons who underwent LCGP dropped dramatically in the first month after surgery, most likely due to reduced ghrelin secretion from the cells localized at the gastric fundus. Ghrelin is supposed to be the principal hunger hormone. The diminished ghrelin secretion resulted probably as a consequence of the blood flow reduction on the gastric wall, secondary to high pressure induced by the edema of the folds. But in our study ghrelin maintain a descending trend even after edema resolution, the median levels at three months are fourth time the base value.

Actual knowledge place the ghrelin's producing cells inside the oxyntic glands, situated near the basal membrane, adjacent to arterial blood flow, most of these cells don't have contact with the gastric lumen (11). Once the gastric wall edema disappeared the hunger sensation increased his level during the follow-up, reaching the highest level at 12 months, but not reaching the initial level before the surgery.

Satiety sensation was rapidly installed due to reduced gastric volume following the plication and the edema of the folds, especially during the first month when the edema was at his peak. Same way as the hunger sensation, once the gastric reservoir increased his volume due to edema resolution and due to pressure resulted from the continuously increased amount of the food intake, satiety sensation was installed slower.

Restrictive bariatric procedure induced a EWL mainly to reduced gastric volume and reduced food intake, but once the gastric volume is increased as an adaptation of larger and larger meals the slop of EWL decrease. Regain can be a possibility. The motivation and the cooperation of the obese patients become essential in maintaining the EWL. Others keys elements in the EWL after LGCP are the hormonal changes promoted by the reduced gastric volume, the reduced appetite induced by the lower ghrelin levels and the increase in the metabolism, these changes maintain the EWL and resolve some comorbidities that are associated with the obesity.

CONCLUSION

LGCP is a new bariatric procedure that it seems to be safe and effective in the short-term results. It could be more attractive, particularly in developing countries, because of a lower morbidity, lower cost and equal efficacy compared with other restrictive procedures. We believe that LGCP could be an acceptable option especially for patients with BMI under 40kg/m^2, who refuse an irreversible procedure such as sleeve gastrectomy. Postprandial plasma levels of ghrelin are decreased at 1 and 3 months after the LGCP, a finding that has also been noted after other bariatricoperations such as sleeve gastrectomy, vertical banded gastroplasty and biliopancreatic diversion with duodenal switch (12,13).

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STUDIU PROSPECTIV PRIVIND PLICATURAREA LAPAROSCOPICA A MARII CURBURI – EFECTELE ASUPRA PIERDERII IN GREUTATE SI A NIVELULUI HORMONILOR DE FOAME

REZUMAT

Introducere: Plicaturarea laparoscopica a marii curburi (LGCP) nu necesita introducerea dispozitivelor implantabile, nici rezectia gastric sau intestinala sau bypass intestinal, iar unul dintre cele mai importante avantaje ale acestei tehnici este potential reversibilitate; de asemenea, poate fi urmata de procedure extensive. Scopul studiului nostru a fost de a evalua pierderea in greutate (excessive weight loss-EWL) si modificarile nivelului ghrelinei pe un lot de pacienti obezi care au fost supusi LGCP.

Metode: Acesta este un studiu prospectiv, ne-randomizat si a inclus toti pacientii operati prin LGCP in cursul anului 2012 in Sectia II Chirurgie a Spitalului Judetean de Urgenta Timisoara. Datele au fost colectate din fisele pacientilor, chestionare si din consulturile de urmarire de la 1, 3, 6 si 12 luni. Toti pacientii au fost prezenti la consultul de control de la 12 luni. Interventia chirurgicala majora a fost considerata LGCP. Principalul rezultat a fost pierderea in greutate (EWL), iar efectele secundare au fost: nivelul ghrelinei, modificarea senzatiei de satietate si foame, cresterea in greutate, complicatiile chirurgicale, spitalizari ulterioare, datele intra-operatorii – durata interventiei chirurgicale, pierderea sanguina.

Rezultate: 14 pacienti au fost inclusi in studiu – supusi interventiei LGCP, 12 pacienti de sex feminin si 2 de sex masculin. Toate procedurile au fost efectuate exclusiv laparoscopic. La interval de 1 luna, EWL a fost – 25,7%, la 3 luni – 34,9%, la 6 luni – 44,9%, iar la 12 luni – 57,6%. Inaintea interventiei chirurgicale, nivelul mediu al ghrelinei a fost 283,05 pg/ml, iar dupa interventia chirurgicala a scazut la 160,60 pg/ml la interval de 1 luna si la 65,40 pg/ml la 3 luni. Comorbiditatile au fost solutionate la 4 din 8 pacienti. Au existat si doua complicatii minore – greturi si varsaturi, care au prelungit spitalizarea si au necesitat tratament conservator. Doi dintre pacienti au prezentat o scadere redusa in greutate datorat lipsei motivatiei.

Concluzie: LGCP poate fi o procedura bariatrica de succes, efectuata de chirurgi bariatrici instruiti, pe un lot de pacienti selectionati, cu motivatie puternica. EWL este satisfacator; complicatiile sunt rare si usoare, necesitand de obicei doar tratament conservator.

TELOMERE LENGHT CHANGES IN ALZHEIMER DISEASE

GROZA SABINE[1], ANGHEL SIMONA[1,2], MIRABELA CRISTEA[2], CARMEN TATU[1,2], GABRIELA TANASIE[1,2], CARMEN PANAITESCU[1,2], OANA GAVRILIUC[1], VIRGIL PAUNESCU[1,2], FLORINA BOJIN[1]

Department of Functional Sciences, "Victor Babes" University of Medicine and Pharmacy Timisoara
Immunophysiology and Biotechnologies Center, Clinical Emergency County Hospital Timisoara

ABSTRACT

Telomeres are regions of repetitive DNA at the end of eukaryotic chromosomes, which prevent chromosomal instability. Telomere shortening in peripheral blood mononuclear cells (PBMCs) has been associated with biological age and several chronic degenerative diseases. Telomere shortening is linked to age- related disease including Alzheimer's disease (AD) and has been reported to be reduced in leukocytes of AD patients. The aim of the present study was to measure telomere length in lymphocytes of patients with AD compared to healthy subjects. We used a new flowcytometric method of hybridization, which provided data referring to relative telomere length compared to control cells. Our data show significant shorter telomere length in AD patients (22.95%) compared to controls (55.49%). In conclusion, the telomere length is age-dependent in lymphocytes and decreased in AD patients, which could mean that the AD pathology may contribute to telomere length shortening. The high variability of telomere lengths in individuals suggests that it will not be useful as a general biomarker for AD. However, it could become a biomarker in personalized long-term monitoring of an individuals' health.

Key words: Alzheimer disease (AD), telomere length, flowcytometric hybridization, lymphocytes

INTRODUCTION

Prior to mitotic cell division, all cellular DNA is duplicated by the action of a DNA polymerase. However, this enzyme does not replicate the very ends of the chromosomes, the so-called telomere region. As a consequence, the telomeres get shorter after each cell division. In normal somatic cells, this shortening contributes to cellular senescence and the telomeres act as a "mitotic clock". Telomeres in all vertebrates are composed of a sequence of six nucleotides (TTAGGG) repeated from a few hundred to several thousand times. The length of telomeres is species-specific and ranges from 5-20 kilobases in man to 20-150 kilobases in mice. Telomeres are considered to have a protective function, keeping the chromosome ends intact, and thereby protecting the underlying genes and avoiding fusion of chromosome ends. Some specialized cells maintain their capacity to divide. Examples are germ cells, fetal cells, haematopoietic stem cells, and basal cells of the epidermis. These cells overcome the ageing problem by the action of the enzyme, telomerase (1). Telomerase maintains telomere length by adding hexameric repeats to the telomeric ends of the chromosomes, thus compensating for the continued shortening of telomeres that would otherwise occur. The length of the telomeres depends both on the age of the cell donor and on the number of times the cell has divided. However, all normal diploid cells are "mortal" and have a limited capacity to proliferate in culture. In contrast, established tumor cell lines can divide forever and are "immortal" mainly due to reactivation of telomerase. Almost 90% of human primary tumors express telomerase, while the cells of most normal tissues lack this enzyme activity. Recently, the hypothesis that the activation of telomerase is necessary for extension of the life-span of human cells by avoiding telomere shortening has been proven (1). Telomerase activity and the preservation of telomere length are, therefore, important for the cancerous process and for the sustained growth of most solid tumors both in vivo and in vitro.

The telomere length can be measured by quantitative FISH in both human (2) and other vertebrate cells (3). Chromosome-specific factors regulate the length of the individual telomere. Short telomeres on the human chromosome 17p might be responsible for the frequent loss of 17p alleles in human cancer, e.g. the tumor suppressor gene for the p53 protein (4). The genetically determined variation in telomerase activity between individuals (5) makes telomere length measurements relevant for the study of age-related diseases. The average telomere length of chromosomes in a cell population can be estimated by quantitative FISH in flowcytometry (6, 7).

Alzheimer's disease is the most common cause of dementia in the elderly, and is characterized by the extracellular deposits of amyloid plaques and intracellular neurofibrillary tangles (8,9). Advancing age is the major risk factor for the development of Alzheimer's disease (10) and overall prevalence of Alzheimer's disease is 14 times higher after 85 years of age compared to 65–69 years of age. Despite the close intimacy between ageing and Alzheimer's disease, very limited knowledge is currently available on this aspect. At the molecular level, ageing is associated with the accumulation of nuclear DNA damage (11,12) and a variety of studies have provided evidence for accumulation of nuclear DNA damage and activation of DNA damage checkpoints (such as apoptosis and senescence) in ageing tissues (13).

Telomere shortening represents a cell intrinsic mechanism for accumulation of DNA damage that may increase the sensitivity of neurons towards oxidative stress and proteotoxicity. Further it has been reported that oxidative stress can increase telomere shortening (14). A number of studies have documented telomere shortening from different organs during ageing (15,16). However, in agreement with very limited cell division, telomere shortening

Received 10th of March 2014.Accepted 25th of May 2014. Address for correspondence: Sabine Groza, MD, PhD student, Department of Functional Sciences, Immunology, "Victor Babes" University of Medicine and Pharmacy Timisoara, EftimieMurgu Square No. 2A, RO-300041, Timisoara, Romania, Phone/fax: + 40 256490507; email: sabine_groza@yahoo.com

was not observed in whole human brain samples (17). Similar studies on patients with Alzheimer's disease revealed divergent results, while telomere shortening was accelerated in peripheral blood cells (18), opposite results were obtained from neurons (19). Telomeres shorten as a consequence of cell division and limit the replicative potential of cells (17). In the adult mammalian brain, glia but not neurons can divide, although active neurogenesis takes place in the subventricular zone of lateral ventricles and the subgranular zone of the dentate gyrus (20). According to this paradigm, age-dependent telomere shortening and decrease in adult neurogenesis could represent a predisposing factor for Alzheimer's disease progression.

Apart from adult neurogenesis, telomere shortening could also alter the proliferative capacity of microglia and inflammatory signaling in the ageing brain. Studies on fibroblasts have shown that senescence induces an aberrant secretion of pro-inflammatory cytokines (21) and similar observation was also made from ageing telomere dysfunctional mice (22).

Despite the intimate association of ageing with telomere shortening and Alzheimer's disease development, its functional relevance on Alzheimer's disease progression has not been fully investigated. The aim of the present study was to measure telomere length in lymphocytes of patients with AD compared to healthy subjects, and to conclude whether immune cells dysfunction can induce changes in inflammatory mediators release, thus contributing to disease maintenance and progression.

MATERIALS AND METHODS

We used peripheral blood mononuclear cells (PBMCs) harvested from Alzheimer disease (AD) patients (n=30) and control patients (n=30). The age of patients with Alzheimer disease ranged from 57 to 84-year old, while the control samples were harvested from elder subjects with similar age range, with no prior diagnostic of Alzheimer disease or other forms of dementia. After signing the Informed Consent form (elaborated based on a well-established protocol, approved by the Ethics Committee of "Victor Babes" University of Medicine and Pharmacy Timisoara), we proceddeed to further analysis.

10 ml of total peripheral blood was used for separation of PBMCs from both AD and control subjects. Peripheral blood was harvested on anticoagulant (Heparin), further diluted with Dulbecco's Phosphate Buffered Saline (PBS; Sigma-Aldrich Company, St. Louis, USA) with a dilution ratio of 1:1, and then we used gradient centrifugation (Ficoll-Hipaque, GE Healthcare Bio-Sciences AB) for 25 minutes at 2500 rpm for peripheral blood mononuclear cells (PBMCs) separation. All PBMCs samples were cryopreserved until further use.

For determination of telomere length of both study and control patients groups, we used Telomere PNA Kit/FITC for Flow Cytometry (Dako), which provides a convenient method for measuring telomeric sequences in vertebrate interphase hematopoietic cells, according to the manufacturer instructions detailed below. Instead of 1301 cell line (tetraploid), we used K562 as control cell line (triploid) and we adjusted the DNA index accordingly (DNA index = 1.5).

In addition to the fluorescein-conjugated peptide nucleic acid (PNA) probe in hybridization solution, the kit contains hybridization solution without probe for correction of cell autofluorescence, wash solution for post-hybridization washes and DNA-staining solution for identification of $G_{0/1}$-cells. In a mixture of sample cells and control cells, the sample DNA is denatured at 82 °C for 10 minutes in an Eppendorf tube in the presence of hybridization solution with or without fluorescein-conjugated PNA telomere probe. Then, hybridization takes place in the dark at room temperature overnight. The hybridization is followed by 2 washes in wash solution at 40 °C for 10 minutes each. Finally the cells are resuspended in DNA-staining solution and stored in the dark at 2-8 °C for 2-3 hours before analysis by flow cytometry. The specific fluorescence from telomere staining will be observed in FL1, and fluorescence from DNA staining will be observed in FL3. Compared with the traditional telomere restriction fragment (TRF) method, a major advantage of the Dako Telomere PNA Kit/FITC assay is that it does not suffer from the interaction of subtelomere sequences.

The probe of the kit recognizes telomeric repeats, but also potential intrachromosomal TTAGGG repeats will be included in the fluorescence signal. In the literature the prevalence of non-telomeric TTAGGG sequences vary (10, 11), but were found to be few and short using the sensitive Q-FISH method with a PNA probe (4). These data and the good correlation with Southern blotting support the idea that intrachromosomal telomere sequences are of less significance for the total hybridization signal detected by flow cytometry (6).

The samples are run in duplicate and that the mean values of the duplicate determinations are used for data analysis. PNA is a synthetic DNA/RNA analog capable of binding to DNA/RNA in a sequence-specific manner obeying the Watson-Crick base pairing rules. In PNA the sugar phosphate backbone has been replaced by a neutral peptide/polyamide backbone keeping the distances between the bases exactly the same as in DNA (8). The neutral peptide backbone gives PNA excellent properties for hybridizing to DNA/RNA. In addition, PNAs are highly resistant to degradation by DNases, RNases, proteinases, and peptidases. The method is optimal for estimation of telomere length, as the fluorescence intensity of the cells is directly correlated to the length of the telomeres (6, 7).

After flow cytometric analysis, the data obtained can be used for determination of a relative telomere length (RTL). The RTL value is calculated as the ratio between the telomere signal of each sample and the control cell (K562 cell line) with correction for the DNA index of G0/1 cells. Sample cells and control cells should are analyzed separately for DNA ploidy in order to be able to make an accurate compensation for the cellular DNA content, according to Vindeløv et al. (12). This correction is performed in order to standardize the number of telomere ends per cell and thereby telomere length per chromosome.

RESULTS

Samples hybridized with the Telomere PNA Probe/FITC should exhibit a fluorescence signal in FL1, which is higher than the background/autofluorescence signal obtained from the sample of the same cells hybridized with the hybridization solution without probe. When analyzing flow cytometry data for cells hybridized with or without the Telomere PNA Probe/FITC it is important only to look at cells in the G0/1-phase of the cell cycle where the cell has one copy of its genome. This is achieved by setting the correct gates. If cells in S or G2/M-phase are not removed by gating, the estimated RTL will not be per genome equivalent.

We analyzed human the PBMCs isolated from blood by gradient centrifugation, and extracted the lymphocytes fraction for telomere length determination. The cells were mixed 1:1 with control cells (K562 cell line). The gated cells were then displayed in the FL3-height versus FL1-height dot plot. We identified cells in the G0/1-phase and set gates around these populations. Statistical data on these cells, together with the DNA index of the cells determined by traditional DNA measurement, were then used for calculation of RTL of the sample cells compared to the control cells in the following way:

$$RTL = \frac{\text{(mean FL1 sample cells with probe - mean FL1 sample cells without probe) x DNA index of } \textbf{control} \text{ cells x 100}}{\text{(mean FL1 control cells with probe - mean FL1 control cells without probe) x DNA index of } \textbf{sample} \text{ cells}}$$

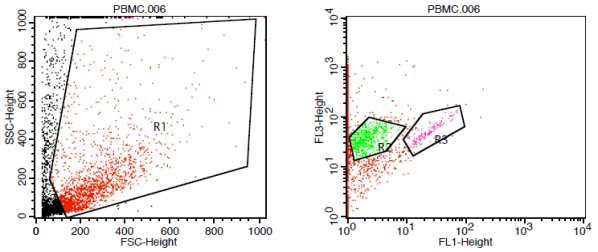

Fig. 1. Dotplot analysis of control subjects' PBMCs without hybridization probe

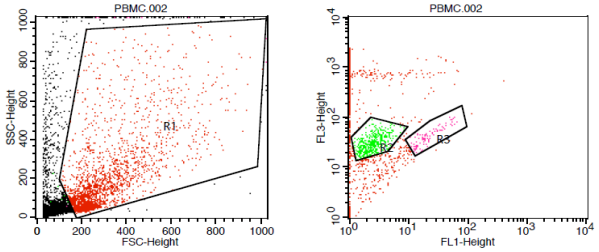

Fig. 2. Dotplot analysis of control subjects' PBMCs with hybridization probe

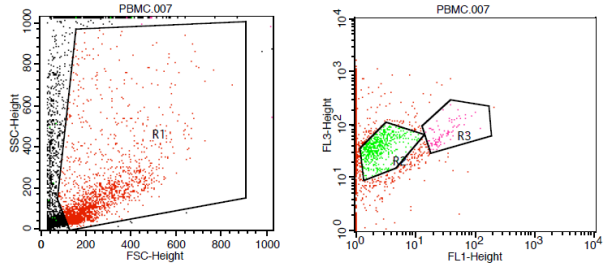

Fig. 3. Dotplot analysis of AD patients' PBMCs without hybridization probe

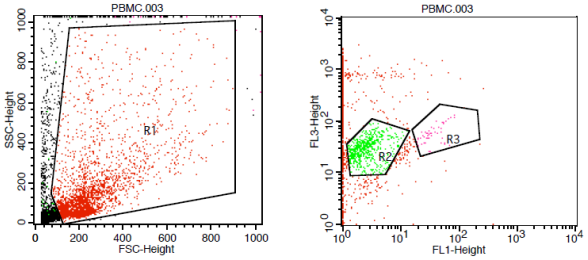

Fig. 4. Dotplot analysis of AD patients' PBMCs with hybridization probe

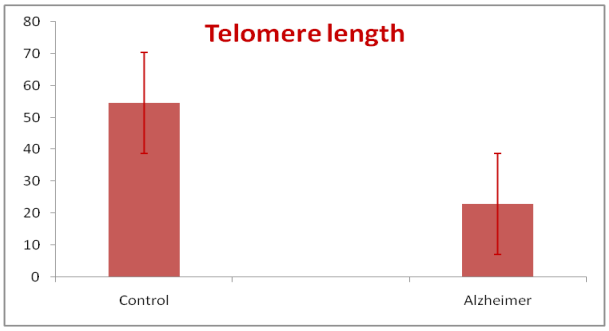

Fig. 5. Statistic analysis of telomere length in control and AD subjects

CONCLUSION AND DISCUSSION

The integrity of the telomere structure and its DNA hexamer (TTAGGG)n repeat sequence is critical for the protection of the ends of chromosomes from degradation and in maintaining overall genomic stability (23,24). The number of DNA hexamer (TTAGGG) n repeats is reduced during each cell division in differentiated cells, and as a consequence telomere length (TL) often decreases in most differentiated cells throughout the lifespan of the organism (23). Shortening of telomeres can result in telomere end fusions and an increased level of chromosome instability (CIN), which is in turn a key initiating event in numerous cancers (including lung, breast, colon, and prostate cancers, as well as certain leukaemia's) (25,26). It has been shown that telomere shortening can be accelerated by environmental factors such as psychological and physiological stress, cigarette smoking, obesity and high homocysteine. Efficiency of TL maintenance is also affected by gender. TL has been shown to be associated prospectively with increased risk of myocardial infarction, coronary artery disease, breast cancer free survival, clear cell renal cell carcinoma survival, post-stroke mortality, dementia and cognitive decline, as well as total survival independent of genetic influences (27-30). The strong association between Alzheimer's disease and ageing suggests that molecular events associated with ageing may promote development of the disease. Given the close intimacy between ageing and telomere shortening, it was important to determine whether telomere shortening would have any direct influence on Alzheimer's disease development and/or progression.

In conclusion, the telomere length is age-dependent in lymphocytes and decreased in AD patients, which could mean that the AD pathology may contribute to telomere length shortening. The high variability of telomere lengths in individuals suggests that it will not be useful as a general biomarker for AD. However, it could become a biomarker in personalized long-term monitoring of an individuals' health.

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MODIFICARILE LUNGIMII TELOMERELOR IN BOALA ALZHEIMER

REZUMAT

Telomerele sunt regiuni de ADN repetitiv situate la capetele cromozomilor eucariotelor, care previn instabilitatea cromozomiala. Scurtarea telomerelor la celulele mononucleare din sangele periferic (PBMC) a fost asociata cu varsta biologica si cu o multitudine de afectiuni cronice degenerative. Scurtarea telomerelor este intalnita in afectiuni precum boala Alzheimer (AD), in care este indusa la nivelul leucocitelor. Scopul prezentului studiu este de a determina lungimea telomerelor la limfocitele pacientilor cu AD comparativ cu subiecti sanatosi de aceeasi varsta. Am utilizat o metoda noua de hibridizare flowcitometrica, care ofera date referitoare la lungimea relativa a telomerelor comparativ cu celulele de control. Datele noastre arata o scurtare semnificativa a lungimii telomerelor la pacientii cu AD (22,95%) comparativ cu subiectii control (55,49%). In concluzie, lungimea telomerelor la celulele limfocitare este dependenta de varsta si este scazuta la pacientii cu AD, ceea ce ar putea insemna ca patologia AD are o contributie la scurtarea lungimii telomerelor. Narea variabilitate inter-individuala a lungimii telomerelor sugereaza ca acesta nu ar putea fi un biomarker general pentru diagnosticul AD. Totusi, lungimea telomerelor ar putea deveni un biomarker personalizat pentru monitorizarea de lunga durata a starii de sanatate in populatie.

Cuvinte cheie: boala Alzheimer (AD), lungimea telomerelor, hibridizare flowcitometrica, limfocite

GADOLINIUM-BASED CONTRAST AGENTS: SAFETY, EFFICACY AND POTENTIAL ADVERSE EVENTS

DELIA MARIA MINCA[1], ELENA LUMINITA ENACHE[2], FABIAN TATU[3], CARMEN TATU[3]
[1]Anatest Laboratory, Timisoara
[2]University of Medicine and Pharmacy of Tirgu Mures
[3] Victor Babes University of Medicine and Pharmacy Timisoara

ABSTRACT

Magnetic resonance imaging is a widely used diagnostic method, allowing for the simultaneous examination of the anatomy and physiological parameters of tissues and organs by measuring the radio-frequency signals emitted by water protons in living tissues. Signal resolution and sensitivity can be further enhanced by adding contrast agents. Nine gadolinium-based contrast agents (GBCAs) are currently approved. The extracellular agents are most widely used in clinical practice and they can be further subclassified into: linear (ionic or non-ionic) and macrocyclic (ionic or non-ionic). GBCAs have been proved to be highly effective and showed favorable clinical profiles. Several minor, transient adverse events were reported, such as headache and nausea, as well as asymptomatic rise in serum iron and bilirubin levels. However, toxic gadolinium ion may be released *in vivo* by transmetallation, a process by which the gadolinium ion is displaced from its ligand by competing endogenous metals: zinc, copper, calcium, and iron. Linear GBCAs were shown to be less stable than the macrocyclic ones under both normal and altered physiological conditions. The most severe complication associated with the use of GBCAs is nephrogenic systemic fibrosis (NSF), which is thought to be caused by the deposition of free gadolinium in tissues of patients with severe renal dysfunction and the subsequent inability of kidneys to eliminate it because of poor water solubility of the gadolinium. Even though nonionic linear GBCAs were proved to trigger this disease in special categories of patients, the use of GBCAs remains safe provided measures are taken to prevent such an event.

INTRODUCTION

Magnetic resonance imaging (MRI) has become a widely used diagnostic method over the past decades due to its ability to produce three-dimensional representations of human tissues with high spatial resolution and contrast, without using harmful radiation (1). MRI allows for the simultaneous examination of the anatomy and physiological parameters of tissues and organs by measuring the radio-frequency signals emitted by water protons in living tissues (2). Signal resolution and sensitivity can be further enhanced by adding contrast agents (CAs), with approximately 35% of MRI scans being performed with the use of CAs (3, 4). Two major categories of CAs are available, paramagnetic and superparamagnetic, each of them being further classified into extracellular, organ-specific and blood pool contrast agents (3). Most widely used paramagnetic CAs contain gadolinium, a rare-earth metal of the lanthanide series; due to its toxicity, the gadolinium (III) ion is administered in the form of chelate complexes, thus preventing the release of metal ion (5).

In MRI, relaxation is the process by which water proton spins are returning back to the equilibrium state once the radio frequency pulse (that disturbed the equilibrium) is turned off (6), the ability of contrast media to increase the relaxation being known as relaxivity (2, 5) . Spin-lattice or longitudinal relaxation (T1) and spin-spin or transverse relaxation (T2) are the two main relaxation processes. T1 and T2 relaxation times of water protons are changed by contrast agents that generate a local fluctuating magnetic field, thus producing changes in tissue contrast (2). Even though all contrast agents shorten both T1 and T2, the difference between them consists in the contrast image they are able to produce, that is positive (T1 contrast agents) or negative (T2 contrast agents) (8).

The Gd(III) ion, with its 7 unpaired electrons, produces a large local magnetic field and it therefore has the strongest effect on the T1 relaxation times (7, 8). However, the free gadolinium ion has cardiovascular and neurologic toxicity, being deposited in liver, bone and lymph nodes (9). For this reason, it is administered in the form of stable chelates, which eliminate its insolubility and toxicity by using a multidentate organic ligand (3, 7, 10). The resulting complex must be highly stable and resistant to the release of free Gd(III) ion through transmetallation (3), a process by which the gadolinium ion is displaced from its ligand by competing endogenous metals: zinc, copper, calcium, and iron (11).

Gadolinium-based contrast agents

Based on their distribution, Gd-based contrast agents (GBCA) are divided into extracellular with renal elimination, extracellular with mixed renal and hepatobiliary elimination and intravascular with renal elimination or blood-pool agents (12), the first group being the most widely used in clinical practice (10). All the nine approved GBCA contain an octadentate co-ligand binding to gadolinium and a single coordinated water ligand (7, 12).

The extracellular agents were the first approved GBCAs

Received 12th of May 2014. Accepted 11th of June 2014. Address for correspondence: Delia Minca, MD, Anatest Laboratory, Inului No.4 Street, Timisoara, Romania, phone/fax: +40256 293192, e-mail: delia.minca@yahoo.com

(12) and they can be further subclassified into: linear (ionic or non-ionic), and macrocyclic (ionic or non-ionic) (10, 13). After intravenous injection, they rapidly distribute in the blood and the extracellular space (12, 13) and are normally eliminated unmetabolized via kidneys through glomerular filtration (13, 14), with half-lives of approximately 1.5 hours in people with normal renal function (5, 12, 13). However, the plasma half-life differs among the available Gd chelates and is dependent upon the volume of distribution and the glomerular filtration rate of the agent, while in patients with impaired renal function is higher (13), thus caution is recommended in these patients.

The extracellular GBCAs do not bind to proteins (10), but three of them (gadoxetic acid disodium, gadobenate dimeglumine and gadofosveset trisodium) were designed as targeted agents (5) and have partial hepatobiliary excretion (10, 13), making them useful for liver imaging (gadoxetic acid and gadobenate) or angiography (gadofosveset) (5, 12,13). The latter binds to human serum albumin and its increased intravascular retention provides greater contrast enhancement (5), although albumin binding is achieved also by the first two of them at a much lesser extent (15).

The first GBCA approved for clinical use in 1986 in Europe and 1988 in the USA and Japan was Gd-DTPA (Magnevist) (7, 16). The nine currently available GBCAs are DTPA (diethylenetriaminepentaacetate) or DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) derivatives (7). They can be classified according to various criteria, including their structure, relaxivity and distribution (10, 11, 13, 14, 15) (Table I).

Table I. Classification of gadolinium based contrast agents and their clinical uses

Contrast agent	Trade name	Structure	Relaxivity	Distribution	Clinical use
Gadopentetate dimeglumine (Gd-DTPA)	Magnevist	Linear, ionic	Standard	Extracellular non-specific	CNS/ whole body
Gadoteridol (Gd-HP-DO3A)	ProHance	Macrocyclic, ionic	Standard	Extracellular non-specific	CNS/ whole body
Gadodiamide (Gd-DTPA-BMA)	Omniscan	Linear, nonionic	Standard	Extracellular non-specific	CNS/ whole body
Gadoterate meglumine (Gd-DOTA)	Dotarem, Magnescope	Macrocyclic, ionic	Standard	Extracellular non-specific	CNS/ whole body
Gadobutrol (Gd-BT-DO3A)	Gadovist, Gadavist	Macrocyclic, ionic	Standard	Extracellular non-specific	CNS/ whole body
Gadoversetamide (Gd-DTPA-BMEA)	Opti-MARK	Linear, nonionic	Standard	Extracellular non-specific	CNS/ whole body
Gadobenate dimeglumine (Gd-BOPTA)	Multi-Hance	Linear, ionic	**High**	Mixed	CNS/ whole body
Gadoxetic acid disodium (Gd-EOB-DTPA)	Primovist, Eovist	Linear, ionic	**High**	Mixed	Liver
Gadofosveset trisodium (Gd-DTPA)	Vasovist, Ablavar	Linear, ionic	**High**	Intravascular	Blood Pool

CNS – central nervous system

According to the approved GBCAs producers, several dosages are recommended, the most common being 0.2 mL/kg (0.1 mmol/kg) body weight (bw); other dosages are to be used for certain products, as follows: 0.12 mL/kg (0.03 mmol/kg) bw for Ablavar/Vasovist, 0.1 mL/kg (0.1 mmol/kg) bw for Gadavist/ Gadovist, 0.1 mL/kg (0.025 mmol/kg) bw and 0.1 mL/kg (0.05 mmol/kg bw when Omniscan is used for the purpose of kidney imaging. Regardless of dosage, all the approved GBCAs are administered by (rapid) intravenous bolus injection at a flow rate of 1 – 2 mL/second (intravenous infusion is also used in case of ProHance), followed by a normal saline flush (5-30 mL, depending on product). The dose is delivered either by manual or power injection and the imaging procedure should be completed within 1 hour after the delivery of contrast agent.

Efficacy and safety of the nine approved GBCAs

Early studies (17, 18, 19) evaluating the safety and efficacy of gadopentetate dimeglumine (Gd-DTPA), the first available GBCA, have concluded it has higher diagnostic value compared to the non contrast-enhanced MRI and exhibits high levels of safety and tolerance. In the multicenter double-blind randomized clinical trial conducted by Russell *et al*. (17), for instance, Gd-DTPA improved diagnostic ability in 65% of patients and led to changes in presumptive diagnosis, along with visualization of more lesions in some of those patients. Adverse reactions in some patients were reported in these early studies, such as asymptomatic rise in serum iron and bilirubin levels, hyper- or hypotension, weakness, conjunctivitis, local burning at injection site, headache, nausea and vomiting, all of which were minor and transient. These adverse reactions were comparable to those related to administration of iodinated non-ionic roentgen contrast media (20).

In a phase III clinical trial (21), gadoteridol yielded more diagnostic information than unenhanced images in more than 70% of patients with suspected intracranial or spinal disease, while the most common adverse reactions were dysgeusia and mild nausea in a minority of cases. Similarly, other studies (22, 23) found gadoteridol is safe and well tolerated by patients and besides the adverse reactions mentioned above, also reported taste perversion and headache (≤1% of patients).

Gadodiamide was shown to be safe and well tolerated in a European multicentre trial (24) in which a range of adverse reactions were recorded in a small number of patients: sensation of heat or coldness, pain or pressure at the injection site, headache, nausea etc. This was in accordance with previous studies (25, 26) reporting mild adverse reactions and minor transient elevations in serum iron. All studies reported gadodiamide injection enhanced visualization of lesions provided more diagnostic information. However, gadodiamide was the first GBCA linked with the development of nephrogenic systemic fibrosis (27), a rare disease that affects patients with renal failure, which will be further discussed.

Postmarketing surveillance studies in Germany (28) and Japan (28) indicate gadoterate meglumine has excellent diagnostic efficacy and favorable clinical safety profile. Mild adverse reactions

were reported in approximately 0.4% of cases and most of them were gastrointestinal in nature.

An integrated analysis of gadobutrol safety conducted by Voth and colleagues (30) based on data from 34 prospective clinical studies concluded in favor of its safety that was well tolerated by all categories of patients, including those with impaired liver or kidney function or cardiovascular disease, with a 4% incidence of minor adverse reactions.

Similarly, gadoversetamide proves to be safe and effective, while the occurrence of adverse reactions following its administration is comparable to other GBCAs (31, 32). In people with renal impairment, however, elimination of gadoversetamide was prolonged, but didn't affect its overall efficacy (32).

A safety assessment report (33) on gadobenate dimeglumine synthesizing data from 65 clinical trials conducted in Europe and USA found generally mild transient adverse reactions occurred, with only 0.2% serious adverse reactions possibly related to the contrast agent.

Gadoxetic acid disodium, as a targeted GBCA, is more effective for radiological diagnosis of liver lesions compared to other imagistic methods, allowing for better detection and localization of such lesions (34, 35). The adverse reactions are the same associated with the use of other GBCAs.

Finally, the only GBCA in the series specifically designed for use in MR angiography (although gadobenate dimeglumine is also successfully used for that purpose), gadofosveset trisodium shows great efficacy and a safety profile similar to all the other GBCAs (36, 37).

Comparisons across the nine approved GBCAs

Many studies have been performed to compare the efficacy and safety of different GBCAs, most of them involving gadopentetate dimeglumine, although intercomparisons of similar contrast agents as regards their structure or clinical use were conducted, as well with iodinated, ferromagnetic or superparamagnetic media. Basically, all the other GBCAs were compared against the first one in the series at some point in phase II or III clinical trials. The results regarding both the efficacy and safety were similar to that obtained with Gd-DTPA for gadoteridol (38, 39) when given in equal doses, although in the earlier, dose comparison study (38) gadoteridol proved to more effective at higher experimental doses. The same efficacy was observed with gadodiamide and Gd-DTPA (40, 41), with significant changes in serum iron 24 h after injection for both GBCAs (40). Similar safety profiles were shown by these two agents and gadoterate meglumine in two multi-centric studies with some mild adverse reactions occurring in relation to each of the contrast agents (42). On the other hand, when compared to the performance of gadobenate dimeglumine (Gd-BOPTA) at an equivalent dose, gadodiamide proved to be inferior in terms of efficacy (43).

Gd-BOPTA and Gd-DTPA have been found to have a similar safety profiles in 10 clinical trials conducted in Europe, United States and Asia over a period of 8 years (44). However, given its higher relaxivity, Gd-BOPTA yields improved diagnostic information for a range of indications, including liver (45), brain (46) or MR angiography (47, 48). A retrospective study (49) comparing reduced dose of gadobenate dimeglumine with standard single dose of gadoterate meglumine showed Gd-BOPTA produced equal or better enhancement in cranial MRI. Again, the high relaxivity of Gd-BOPTA played a critical role in this respect and allowed for the dose reduction.

Nevertheless, when comparing Gd-BOPTA and gadoxetic acid trisodium for liver MRI, the latter showed superior liver enhancement in the overall population, as well as in the cirrhotic subgroup in a phase III clinical trial (50)., which is not surprising since Gd-EOB-DTPA is the only GBCA specifically targeted for liver MRI. Likewise, Gd-EOB-DTPA had significantly higher sensitivity compared to gadopentetate dimeglumine in the detection of small hepatocellular carcinoma (51).

Gadoversetamide was also compared with Gd-DTPA in phase III studies in patients with liver (52) and central nervous system pathologies (53) in which safety, tolerability and efficacy of the two GBCAs were assessed, with no statistical differences observed between the two gadolinium chelates with respect to confidence in diagnosis, level of conspicuity, and border delineation.

Gadobutrol provided higher conspicuity and enhanced detection of cerebral lesions than gadopentetate, both GBCAs being administered either at equal (54) or double dose (55). When compared to gadoterate meglumine in patients with suspected chronic myocardial infarction, gadobutrol showed superior contrast (56).

Comparison of gadofosveset trisodium with gadopentetate dimeglumine (57) or gadobenate dimeglumine (58) in angiography resulted in either same image quality for gadofosveset at standard (0.03 mmol/kgbw)/high doses (0.05 mmol/kgbw) and gadopentetate dimeglumine at increased dosage (≈0.25 mmol/kg), or slightly better performance of gadofosveset over gadobenate dimeglumine.

Nephrotoxicity of GBCAs

Gadolinium chelates are routinely used nowadays in magnetic resonance imaging, mostly for CNS pathology, followed by contrast-enhanced MR angiography, liver imaging, and breast evaluation for malignancy (59). Unfortunately, toxic Gd(III) ions may be released *in vivo* by transmetallation with other metal ions, thus the kinetic stability of the gadolinium chelates is very important (3). Macrocyclic chelates have been shown to be more stable than the linear ones (60, 61). A study (62) on the stability and dissociation rate of the approved GBCAs in human serum under normal physiological conditions (pH 7.4 and 37^0C), as well as in case of elevated serum phosphate levels (often seen in patients with end-stage renal disease) concluded nonionic linear chelates are less stable than the ionic linear ones under normal and particularly under altered physiological conditions, while the microcyclic agents remained stable.

The interference of gadodiamide and gadoversetamide with colorimetric determinations of serum calcium has been well documented (63, 64, 65, 66), the release of gadolinium ion resulting in spurious hypocalcemia (especially in patients with renal insufficiency), which may lead to unnecessary and

potentially inappropriate treatment. Furthermore, transmetallation may occur with other endogenous metal ions, causing both positive and negative interference with other serum assays: zinc, iron, magnesium, copper, angiotensin-converting enzyme and total iron binding capacity (13, 61, 67, 68, 69, 70). Gadolinium deposits were detected in several organs, particularly in the skin, kidney, liver, heart and bones (71, 72, 73). Several GBCAs (namely Omniscan, MultiHance, ProHance and Magnevist) were shown to stimulate dermal fibroblasts proliferation, along with up-regulation of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinases-1 (74, 75). Contrast-induced nephropathy, defined as increase in creatinine concentration (76) or decrease in glomerular filtration rate (77) has been associated with GBCAs, particularly at high doses and in patients with renal impairment. However, in patients with normal renal function, their use remains safe, due to the rapid excretion of gadolinium complex.

But the most severe and possibly life-threatening complication associated with the use of GBCAs is nephrogenic systemic fibrosis (NSF), a fibroproliferative disease affecting people with severe renal impairment which was first observed in 1997 (78). NSF is thought to be caused by the deposition of free gadolinium in tissues of patients with severe renal dysfunction and the subsequent inability of kidneys to eliminate it because of poor water solubility of the gadolinium (79). A recent review by Zou *et al*. (80) indicates dialysis, edema, hyperphosphatemia, epoetin use and proinflammatory conditions as additional factors involved in the onset of NSF.

This causative link between GBCAs and NSF has prompted the USA Food and Drug Administration to request a warning box to be added by manufacturers to the labels of all GBCAs in 2007 (81) stating the increased risk of NSF in patients with acute or chronic severe renal insufficiency (glomerular filtration rate <30mL/min/1.73m2), or acute renal insufficiency of any severity due to the hepato-renal syndrome or in the perioperative liver transplantation period. Subsequently, the European Medicines Agency has released in 2009 a set of recommendations (82) based on the various degrees of risk demonstrated for the approved GBCAs. Hence, for high-risk GBCAs, in addition to the categories of patients in the FDA warning, contraindications were also recommended for newborn babies up to four weeks of age. Additionally, patients with unknown kidney problems should always be screened for renal function and that women should discontinue breastfeeding for at least 24 hours after a scan. For medium- and low-risk GBCAs warnings in the prescribing information concerning their use in patients with severe kidney problems and patients receiving a liver transplant are also recommended, and screening for kidney problems is advised, while decision on breastfeeding discontinuation is to be taken by the doctor and mother.

So what led to this position? As highlighted above, GBCAs were increasingly linked with NSF, while gadodiamide was most frequently incriminated. In 2006 Grobner (83) was the first to suggest the development of NSF after exposure to gadodiamide. Shortly after, Marckmann *et al*. (27) reported 13 cases of NSF after exposure to the same contrast agent in patients with end-stage renal disease. After that, more and more reports were published adding more evidence in support of this link (8, 9, 13, 72, 73, 76, 77). Based on data from literature, Tweedle and colleagues (11) have classified GBCAs into three groups according to their association with NSF, gadodiamide, gadopentetate dimeglumine and gadoversetamide being linked with the greatest number of cases. The macrocyclic GBCAs are the least involved, while the protein-binding ones have low incidence of NSF cases. In a retrospective analysis at two large medical centers over a period of 10 years, Prince *et al*. (84) calculated the incidence and risk factors of NSF for patients receiving standard or high doses of GBCA, as well as in patients with renal impairment. At standard dose used for patients not screened for renal function, no case of NSF was found and an incidence of 0.17% for high-dose usage was recorded. In patients with renal dysfunction, after exposure to high dose GBCAs, the incidence was 0.4% (hemodialysis patients), 8.8% (eGFR<15mL/min, not on hemodialysis) and 19% (acute renal failure and increased creatinine level). This led to the conclusion that, while in the first category of patients hemodialysis may prevent NSF, in the remaining two groups, the risk of NSF after administration of a high dose GBCA is associated with delayed hemodialysis, proinflammatory events, and hyperphosphatemia.

In conclusion, GBCA stability and dosage are important considerations in the selection of the particular agent to be used for MRI scans, especially in renally impaired patients, because of the possibility of the release of free gadolinium ion in the body under certain physiological conditions, with the subsequent deposition that may result in a series of adverse reactions and complications, out of which the most severe is the nephrogenic systemic fibrosis. Even though nonionic linear GBCAs were proved to trigger this disease in special categories of patients, the use of GBCAs remains safe provided measures are taken to prevent such an event.

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AGENȚI DE CONTRAST PE BAZĂ DE GADOLINIU: SIGURANȚĂ, EFICACITATE ŞI EFECTE ADVERSE POTENȚIALE

REZUMAT

Imagistica prin rezonanță magnetic este o metodă de diagnostic larg utilizată, care permite examinarea simultană a anatomiei și a parametrilor fiziologici ai țesuturilor și organelor prin măsurarea semnalelor de radiofrecvență emise de protonii din țesuturile vii. Rezoluția și sensibilitatea semnalelor poate fi sporită prin adăugarea de agenți de contrast. În prezent există nouă agenți de contrast pe bază de gadoliniu aprobați (GBCA). Agenții extracelulari sunt cel mai folosiți în practică și pot fi clasificați în: lineari (ionici sau non-ionici) și macrociclici (ionici sau non-ionici). S-a dovedit că GBCA sunt foarte eficienți și au profiluri clinice favorabile. Au fost raportate unele reacții adverse minore tranzitorii, cum ar fi dureri de cap și greață, precum și creșteri asimptomatice ale fierului seric și bilirubinei. Cu toate acestea, ionul toxic de gadoliniu poate fi eliberat *in vivo* prin transmetalare, un proces prin care ionul de gadoliniu este desprins de ligandul său și substituit de către metale endogene competitoare: zinc, cupru, calciu și fier. S-a demonstrat că GBCA lineari sunt mai puțin stabili decât cei macrociclici atât în condiții fiziologice normale, cât și alterate. Cea mai gravă complicație ca urmare a utilizării GBCA este fibroza sistemică nefrogenă (NSF). Se crede că NSF este cauzată de depunerea gadoliniului în țesuturile pacienților cu disfuncție renală severă și de incapacitatea rezultată a rinichilor de a-l elimina, datorită solubilității scăzute a acestuia. Deși s-a demonstrat că GBCA lineari nonionici declanșează această boală la anumite categorii de pacienți, utilizarea GBCA rămâne sigură în condițiile în care se iau măsuri pentru prevenirea acesteia.

REGULAR PHYSICAL ACTIVITY AND ABLATION OF LONE ATRIAL FIBRILLATION OUTCOMES IN YOUNG PATIENTS

ALINA G. NEGRU[1, 2], GABRIEL IVĂNICĂ[1], MARIUS ANDRONACHE[3], ADINA BUCUR[4], ŞERBAN NEGRU[2], ADRIAN IVĂNICĂ[5], C.T. LUCA[1,2], ADINA IONAC[1,2], LUCIAN PETRESCU[1,2], ŞTEFAN I. DRĂGULESCU[1,2]

[1]Timişoara Institute of Cardiovascular Medicine
[2]Department of Cardiology, University of Medicine and Pharmacy "Victor Babeş" Timişoara
[3]CHU Nancy Rythmologie Cardiaque
[4]Departament of Public Health and Health Management, University of Medicine and Pharmacy "Victor Babeş", Timişoara
[5]Robert-Bosch-Krankenhaus Kardiologie

ABSTRACT

Introduction: Over the years it has been shown that sports reduce the risk of heart disease, stroke, metabolic syndrome, diabetes mellitus, hypertension, cancer, depression and other pathological conditions. At the same time there is growing evidence that long term sustained athletic activity is associated with atrial fibrillation (AFIB). Until present there is no evaluation of the AFIB ablation outcomes in leisure time sport practitioners compared to sedentary young patients. **Aim of the study:** The present study aims to evaluate mainly the association between regular physical activity and AFIB ablation outcomes in non-professionals (excluding endurance athletes or other professional sports practitioners) compared to sedentary patients in terms of peri-procedural and long term success ablation success. **Methods:** The study population consisted of 173 young patients (p) diagnosed with lone AFIB, admitted for the AFIB ablation and divided in two groups- 88 p performing moderate to vigorous physical activity and 87p not implicated in any sports activity. Mean age was 41±4. AFIB ablation was performed in all patients included after the completion of a personalized questionnaire, physical examination, ECG, echocardiography (for LA volume, EF), blood testing of each individual. The patients were included during 36 months; the follow-up period for each patient was 12 months while the subjects were evaluated for early and late recurrences, need for antiarrhythmic treatment in order to maintain sinus rhythm. Other correlations between LA volume, total procedure time, age and relapse were performed. **Results:** The percentage of men practicing sports and diagnosed with lone AFIB (76.1%) is significantly higher than the number of women included in the same group (23.9%). On the other hand, in the sedentary patients, the percentage of men is even greater (10-fold) than the number of women compared to the sports group. However, the gender does not play a role in the rate of recurrences. There was a percentage of 50% from the athletic group and 67% from the non-athletic group that necessitated antiarrhythmic treatment for a maximum of 3 months after ablation in order to maintain sinus rhythm. There was no significant difference in the scores for antiarrhythmic medication group and no medication group in terms of recidive t (84) =-1.3, p=0.2. Atrial fibrillation recidive for both groups was evaluated at 9% (11.5% from the physical activity group and 7.5% from the sedentary group). As for the scores for LA volumes (LA volume reference=90 ml), there was noted no difference either, t (84) =1, p=0.3. Significantly data were obtained by the evaluation of the total procedure time in patients that received peri-procedural antiarrhythmic medication; the procedure was clearly shorter in patients under anti-arrhythmic medication. A very important outcome was that compared with non-exercisers, the patients with regular physical activity had 35% lower risk of AFIB recidive after the first procedure (P=0.02) (early and late recidive).

Conclusions: Our study data supports a statistically significant association between regular physical activity and lower incidence of recurrences post AFIB ablation in non-professional physical activity performers. However the question how much is too much is still to be answer.

Key words: atrial fibrillation ablation, non-professional athletes, physical activity, exercise, ablation outcomes.

INTRODUCTION

Atrial fibrillation (AFIB) is considered the most common arrhythmia and its prevalence tends to increase along with aging of the population from 0.7% at younger ages to 17.8% around 80 years (1). The same study (the Rotterdam study), revealed the prevalence and incidence as greater in men than in woman. An older study dating from 1994 revealed a prevalence of 0.5% below 40 years and 8% in the patients with ages over 80 years (2). All this data was obtained from the general population. Several studies have reported higher rates of AF among professional athletes. Current practice of sport with a lifetime greater than 1500h was associated with lone atrial fibrillation, the proportion of patients found in this study was greater than double (31%) compared to the proportion observed in controls (14%) (3). Although the presence of AF in athletes has been discussed and described previously (4,5), actually there is poor data availability and inconsistent information regarding the association of regular physical exercise with atrial fibrillation (excluded the professional athletic activity and endurance sports) (6).

AIM OF THE STUDY

This is the first study that aims to evaluate the evidence of the association between regular physical activity and the outcomes of

Received 12th of May 2014. Accepted 15th of June 2014. Address for correspondence: Alina G. Negru, MD, "Victor Babeş" University of Medicine and Pharmacy Timişoara, No. 2 Eftimie Murgu Square, Timişoara, phone: +40-746-239777, e-mail: eivanica@yahoo.com

atrial fibrillation ablation especially the efficiency of atrial fibrillation ablation in non-professional athletes or exercisers compared to non-exercise population in terms of early and late relapse.

METHODS

It is a prospective, case control study in patients undergoing AFIB ablation. Professional athletes were excluded. The study duration was 3 years (36 months) and the follow-up period after ablation was 12 months for each patient.

Lone atrial fibrillation is a term that was first introduced in 1954 by Evans and Swan (8). Currently, lone atrial fibrillation is considered when clinical and echo evidence of cardiovascular or pulmonary disease has been excluded. Pathologic conditions such as hypertension, diabetes, hyperthyroidism, acute infections, recent cardiothoracic or abdominal surgery, and systemic inflammatory diseases must be also excluded in order to diagnose the lone AFIB (9).

Study population.

We included in the study a number of 173 from 210 initial candidates for inclusion, with lone atrial fibrillation, selected for atrial fibrillation ablation and filtered by the baseline information. Participants were only included after signing the informed consent form and after the completion of a questionnaire containing information about: the type of sport, number of hours spent training, medical history, lifestyle, cardiovascular risk factors. Other baseline evaluation consisted of standardized physical evaluation, electrocardiogram (ECG), echocardiography with evaluation of the left atrial volume and size and of the ejection fraction (EF), blood tests including thyroid hormones FT3, FT4 and TSH.

We divided the patients in two equal groups:

The first group included 87 patients with the following characteristics: age below 45 years; sedentary (patients which are not performing any type of controlled regular physical activity); diagnosed with lone AFIB (at least two separate documented episodes of AFIB); EF >55%; without associated pathologic conditions; with or without anti-arrhythmic medication; admitted in the hospital for the catheter ablation of the AFIB.

The 88 patients included in the second group matched the following criteria: age below 45 years; moderate to vigorous regular physical exercise performed between 3 and 7 times per week; presence of lone AFIB (minimum two separate documented episodes of AFIB); EF>55%; without any associated pathologic conditions; with or without anti-arrhythmic medication; option for radiofrequency catheter ablation (RFA).

Physical activity assessment

The exercise was assessed by level of intensity. We included in the exercise group all the patients that fulfilled the above mentioned criteria and practiced moderate or vigorous physical activity of minimum 3 times per week, for 1 hour. The physical activities were categorized as moderate or vigorous on the basis of current knowledge of the overall level of intensity required for the average person engaged in it, taking into account brief episodes when the level of intensity required for the activity might increase or decrease considerably[10].

We defined as moderate activity in accordance with CDC and ACSM Guidelines all the activities between 3.0 and 6 METs (3.5 to 7 kcal/min) [11]. The patients classified in the moderate activity group were performing the following activities based on the completed questionnaire:

- race walking less than 8 km/h
- bicycling 8 to 14 km/h, level terrain or with a few hills
- stationary bicycling using moderate effort
- recreational swimming

We defined as vigorous activity [11]all the activities greater than 6.0 METs (more than 7 kcal/min). The patients classified in the vigorous activity subgroup were performing the following activities:

- race walking and aerobic walking 8 km/h or faster
- jogging or running
- bicycling more than 16 km/h or bicycling on steep uphill terrain
- stationary bicycling using vigorous effort
- swimming steady paced laps

Study design

The patients were included in the study for 36 months and the follow-up period was 12 months for each patient, divided in 4 visits every 3 months.

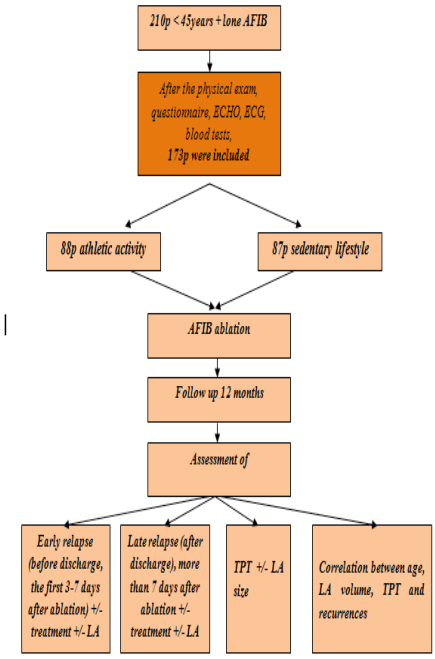

Fig.1. Study design. AFIB-atrial fibrillation ablation, ECHO- cardiac echocardiography, ECG- electrocardiogram, LA- left atrium, TPT-total procedure time.

Catheter ablation procedure

Pulmonary vein isolation was performed using a CARTO 3 imaging system after the integration of an atrial computer tomography. The 3D reconstruction and mapping of the pulmonary veins were obtained using a ten pole Lasso type catheter and an irrigated NaviStar ThermoCool catheter (Biosense Webster). The Lasso catheters were installed via an 8 F sheath (Mullins fixed curve, St. Jude) and the NaviStar catheter was passed in the left atrium using fluoroscopic guidance through the same puncture point as the Lasso. During the entire procedure heparin was injected in order to maintain an activated clotting time between 300 and 400s. Antral pulmonary vein ablation was performed for all veins in each

patient. The bipolar electrograms were filtered at 50-1000Hz and displayed at 100 mm/s sweep speed (Cardiolab System, Prucka Engineering). Radiofrequency energy was applied using a Stockert generator (Biosense Webster). The irrigation was between 17 and 30 ml/min with saline solution 0.9%. The temperature and power limits were 45ºC and 35W for the pulmonary veins and left atrium and 20ml/min and 20-25W for the interior of the coronary sinus.

Post-ablation management

After the procedure the heparin was stopped and the low-molecular-weight heparin was administered concomitant with dabigatran for 24h or with acenocumarol until the INR reached the therapeutic range between 2 and 3. The anticoagulation therapy was stopped after a minimum of 3 months in patients free of AFIB recurrences. The antiarrhythmic therapy was used in most cases during the first 3 months after ablation in order to stabilize to sinus rhythm and totally interrupted after the mentioned blanking period. The patients were evaluated at 1 month, 3 months, 6 months and 12 months after ablation by physical examination, ECG, ECG Holter/24 h or 7 days depending of the described symptoms, cardiac echocardiography.

RESULTS

Statistical analysis was performed using the SPSS program. There was observed a male predominance between both groups (physical activity and sedentary groups). From the 88p included in the athletic activity group there were a number of 67 men (76.1%) and 21 women (23.9%), as for the 87 patients included in the sedentary lifestyle group there were a number of 79 men (91%) and 8 women (9%) This finding may suggest on one hand that the young population diagnosed with lone AFIB consists mainly of men which can be ten times more numerous than women in the sedentary group of patients. However, the sex does not play a role in the rate of recurrences which seems similar in women and men.

The peri-procedural and up to three months antiarrhythmic treatment was necessary in 102p from 172 to stabilize to sinus rhythm. After the 3 months treatment, the antiarrhythmic was withdrawn. There were a number of 44 p (50%) from the athletic group and a number of 58p (67%) from the non athletic group that has necessitated antiarrhythmic treatment, showing the clear numeric superiority of the sedentary individuals that necessitated medication after the AFIB ablation.

The total number of relapse to AFIB for both exercisers and non-exercisers was 33 (9%).

From a total number of 86p performing physical activity, there were 13p (7.5%) with AFIB relapse at minimum 6 months. The relapse among the 87 sedentary patients was 20p (11.5%). This may suggest that sport can play a role in the outcome of the ablation procedure in the sense of increasing the chances of success in patients with lone AFIB which perform physical activity. This is a very important end-point of our study.

	No. relapses	% P
Total	33	19%
Exercise individuals	13	7.5%
Non-exercise individuals	20	11.5%

Fig. 2. Relapse table showing the numbers of recurrences in the exercise or non-exercise groups and the percentage (%P) from total number of patients included in the study, corresponding to each number of recidives.

The Levene's test for the equality of variances showed that the variability in all couples of groups that we compared was about the same. We compared several groups of patients in terms of procedural success translated by recidive evaluated at 6 months: patients with and without antiarrhythmic medication for the first 3 months after the ablation, patients with left atrial volume below and above 90 ml, respectively patients with left atrial volume below and above 120ml.

For the first two groups with/without antiarrhythmic medication we obtained the following data. As mentioned above, there were a total number of 86 patients undergoing moderate or vigorous exercise. The late recidive among exercisers was independent of the presence or absence of the antiarrhythmic treatment that was administered in some cases for a maximum of 3 months after ablation.

An independent samples t-test was conducted to compare recidive in the group of young patients sport practitioners and sedentary which were divided in medicated and non-medicated after the ablation procedure. There was not a significant difference in the scores for antiarrhythmic medication group (M=0.3, SD=0.45) and no medication group (M=0.17, SD=0.4), in terms of recidive; t(84)=-1.3, p=0.2.

For the second couple of groups, with left atrial volume smaller or greater than 90 ml, we obtained the following data: from a total number of 86 patients recidivated, 61 had a left atrial volume greater than 90 ml and 25 patients had a value of the left atrium volume smaller or equal to 90ml. However, there was no significant difference in the scores for left atrial volume under 90 ml (M=0.16, SD=0.4) and above 90 ml (M=0.26, SD=0.44); t (84) =1, p =0.3. These results suggest that the left atrial size does not affect the outcome of the AFIB ablation regarding the recidive at 6 months.

The third couple of groups compared for recidive were patients (mixed population of exercisers and non-exerciser) with left atrial volume under 120 ml (36p) and above 120 ml (50p). The conducted t-test, again, showed no significant difference between the two groups- the group with left atrial size above 120 ml (M=0.25, SD=0.43) and the group with left atrial size under 120 ml (M=0.22, SD=0.41); t (84) =0.32, p=0.7.

A statistically significant data that obtained was from the last couple of groups. This consisted of evaluation of the total AFIB ablation procedure time in patients that had received or not peri-procedural medication. The procedure was clearly shorter in patients under antiarrhythmic medication (M=280, SD=84) compared with no antiarrhythmic medication (M=202, SD=51); t (84) =-5, p=0.001.

One of the most important outcomes of our study already mentioned but need to be reminded, was that compared with no regular exercise, individuals with moderate-intensity or vigorous exercise had 35% lower risk of AFIB relapse after the first ablation (p=0.02).

DISCUSSIONS

The mechanisms by which sports could generate lone AFIB were described before. They include the alteration of the balance between sympathetic and parasympathetic nervous system having as consequence increases in the vagal tone[12] (with bradycardia and shortening of the atrial refractory period) and the left atrial

fibrosis associated with left atrial enlargement as a substrate for micro-reentry circuits (13). There was also incriminated the loss of electrolytes due to sweating, a changing that may modify the electrophysiological properties of the atrial tissue. Coumel (14) studied the influence of autonomic system in the appearance of AFIB and atrial flutter with the specification that AFIB occurs more frequently in men than in women. Looking at our study findings, the number of women with lone AFIB belonging to the athletic group is 3 times greater than the number of women belonging to the sedentary population group. That can suggest that the exercise may be responsible for the vagal mechanism of producing AFIB in both sexes. Regarding this aspect it also should be noted that the inclusion rate of men population is superior of that of women. The limitation of our study regarding this aspect consists in the basic number of males implicated in athletic activity which is known to be greater in general than that of women. Once installed AFIB can be treated conservatory or by catheter ablation targeting the isolation of the pulmonary veins. After performing the AFIB ablation for all the patients included in our study (sedentary and physical activity performers), two interesting findings came out: among the sedentary patients there was a significantly greater number of patients which required antiarrhythmic treatment for the first three months in order to obtain the stabilization to sinus rhythm than in the athletic group; on the other hand, the recidive at 6 months was significantly greater in the athletic group with the specification that after ablation, at 12 weeks, the athletes resumed their activity but at a lower intensity.

For our two groups of patients, the correlation between the recidive and the left atrial volume remained without any signification for the two values that we used as reference (90 ml and 120 ml). However, GIRAFA study data (15) showed that patients with lone atrial fibrillation had a larger atrial size compared with controls. The same study revealed that patients with a first episode of AFIB had the same atrial size compared with those suffering recurrences. The cumulated data from the GIRAFA study and our own study permitted the following conclusion: if the left atrial volume is a predictor for the AFIB incidence, after the ablation procedure the same parameter cannot be used to predict recurrences. The echographic data from another study conducted by Pelliccia et al suggest that structural remodeling due to exercise is often present in the atrium of elite athletes (16). Their data showed that the individuals implicated in regular endurance practice have a larger atrium compared with sedentary controls. Our study which included leisure time physical activity individuals performing moderate or vigorous activity did indeed showed an enlargement in the left atrial volume in this population, significantly higher compared to non-exercisers (61% in athletes compared to 29% in sedentary individuals). However, this outcome does not affect the number of recurrences in our study, suggesting that even there is a difference in the AFIB mechanisms of the two groups, once the ablation is performed there is no influence of the left atrial volume on the peri-procedural or long term success.

Systematizing the data obtained in our study we may conclude that leisure time sport even at moderate or vigorous intensity has not the same effects on the heart as endurance training in terms of AFIB. Even we did not study the incidence but the ablation outcomes we may conclude that long-term endurance training profoundly affects heart and body's physiology. Among other things it is shown to reduce the heart rate and testosterone levels (17,18). Greek researchers evaluated participants in a distance run over a period of 36 hours (19). They observed a 152-fold increase in C-reactive protein levels and an 8000-fold increase in the level of interleukin-6, both important markers of inflammation. The conclusion is that increases at such a scale of the inflammatory markers may induce a potent systemic inflammatory response which at its turn may produce the enlargement of the left atrium with arrhythmic complications. This is another theory of arrhythmogenesis in sport practitioners, the less explored until now.

Regarding the detraining as a mean to prevent AFIB recurrences, there is some evidence proving that the patients who have been ablated for right atrial flutter are at risk to develop atrial fibrillation post-ablation if they have a history(81% increased) and continuation of endurance sports (68% increased risk) (20). Our study revealed very important data regarding this aspect, namely, the outcome that compared with no regular exercise, individuals from the physical activity group had 35% lower risk of AFIB recurrence after the first ablation procedure (early and late recurrence). Those data are contradictory with the actual situation described in the literature, with the specification that our physical activity patients are not endurance professional athletes as the individuals included in almost all published studies. The difference in the intensity and duration of sports activity, lighter in our study population might be the changing point, raising again the question: how much is too much?

CONCLUSIONS

Is exercise good or bad? This is the questions that rise after each study evaluation. Given the fact that through our study we did not evaluate the incidence of the AFIB in the included individuals, but the outcomes of the AFIB ablation, the answer at this question might not be a direct one. All the patients included in our study had been already diagnosed with AFIB. However, the most important outcome was that compared with non-exercisers, the individuals included in the physical activity group had 31% lower risk of AFIB relapse after the first ablation procedure (early and late recurrences included). This suggests that leisure time physical activity performed in moderate or vigorous way has a great impact on the ablation success even in the conditions of continuation of sports after the procedure (at lower intensity than usual). Another important outcome is that the antiarrhythmic medication given peri-procedural shortens the total procedure time and stabilizes to sinus rhythm and does not influence the rate of recurrences. On the other hand, there is no influence of the left atrial volume, gender, age on the ablation success.

Overall, our data support a statistically significant association between regular physical activity and lower incidence of recurrences after the AFIB ablation procedure in leisure time physical activity performers.

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ACTIVITATEA FIZICA REGULATA SI REZULTATELE ABLATIEI FIBRILATIEI ATRIALE LA PACIENTII TINERI

REZUMAT

Introducere: De-a lungul anilor a fost dovedit ca exercitiul fizic reduce riscul aparitiei bolilor cardiovasculare, a accidentelor cerebrale, sindromului metabolic, diabetului zaharat, hipertensiunii, cancerului, depresiei si a altor patologii. In acelasi timp, exista dovezi ca activitatea fizica pe termen lung este asociata cu fibrilatia atriala (AFIB). Pana in prezent nu exista o evaluare a rezultatelor ablatiei AFIB la cei care efectueaza efort fizic moderat, comparativ cu persoanele tinere sedentare. **Scopul studiului:** Studiu prezent isi propune in principal evaluarea asocierii dintre activitatea fizica regulata si rezultatele ablatiei AFIB la sportivii neprofesionisti (excluzand sportivii de anduranta sau alte tipuri de sportivi de performanta) comparativ cu pacientii sedentari in ceea ce priveste etapele peri-procedurale si succesul pe termen lung al ablatiei. **Metode:** Populatia de studiu a fost formata din 173 de pacienti tineri (p) diagnosticati cu AFIB, spitalizati pentru ablatia AFIB si impartiti in doua grupuri – 88 p care efectuau activitate fizica moderata sau intensa si 87 p care nu erau implicati in activitati sportive. Media varstei a fost 41±4. Ablatia AFIB a fost efectuata la toti pacientii inclusi in studiu dupa completarea unui chestionar personalizat, examen clinic, ECG, ecocardiografie (pentru volumul AS, FE) si teste hematologice ale fiecarui individ. Pacientii au fost inclusi in studiu pe o perioada de 36 de luni; perioada de urmarire a fiecarui pacient a fost de 12 luni, timp in care pacientii au fost evaluati pentru aparitia recurentelor precoce si tardive, necesitatea tratamentului antiaritimic pentru mentinerea ritmului sinusal. Au fost efectuate si corelatii intre columul AS, timpul total necesar procedurii, varsta si recaderi. **Rezultate:** Procentul persoanelor de sex masculin care practica sport si au fost diagnosticati cu AFIB (76,1%) a fost semnificativ mai crescut comparativ cu cel al persoanelor de sex feminin incluse in acelasi grup (23,9%). Pe de alta parte, la pacientii sedentari, procentul persoanelor de sex masculin este chiar mai mare (de10 ori) decat cel al femeilor comparativ cu grupul care practica sport. Totusi, sexul pacientilor nu joaca un rol in aparitia recurentelor. A existat un procent de 50% din grupul atletic si 67% din grupul non-atletic care a necesitat tratament anti-aritmic pentru o durata de maxim 3 luni dupa ablatie pentru mentinerea ritmului sinusal. Nu a existat o diferenta semnificativa intre grupul cu medicatie anti-aritmica si cel fara medicatie anti-aritmica in ceea ce priveste recidiva, t (84) =-1.3, p=0.2. Recidiva fibrilatiei atriale pentru ambele grupuri a fost evaluata la 9% (11,5% pentru grupul cu activitate fizica si 7.5% pentru grupul sedentar). In ceea ce priveste volumul AS (volum de referinta AS =90 ml), nu a fost determinata nici o diferenta, t (84) =1, p=0.3. Date semnificative au fost obtinute prin evaluarea timpului total necesar procedurii la pacientii care au fost tratati cu medicatie anti-aritmica peri-procedural; procedura a fost de durata mai scurta la pacientii cu medicatie anti-aritmica. Un rezultat foarte important a fost ca pacientii cu activitate fizica regulata au avut un risc de recidiva a AFIB dupa prima procedura cu 35% mai scazut decat pacientii sedentari (P=0.02) (recidiva pe termen scurt si lunge).
Concluzii: Datele studiului nostru sustin asocierea semnificativa statistic intre activitatea fizica regulta si scaderea incidentei recurentelor post-ablatia AFIB la sportivii ne-profesionisti. Totusi, ramane de stabilit cat de mult este prea mult.
Cuvinte cheie: ablatiafibrilatiei atriale, sportivi neprofesionisti, efort fizic, efectele ablatiei

BCL-2 EXPRESSION AS A NEGATIVE PROGNOSTIC FACTOR IN HODGKIN LYMPHOMA

POTRE-ONCU OVIDIU, IONITA IOANA, IONITA MIHAI, CALAMAR DESPINA, SORICA CRISTINA, IONITA HORTENSIA

"Victor Babes" University of Medicine and Pharmacy Timisoara, Romania

ABSTRACT

Introduction: Although important progresses have been made in Hodgkin's disease (HD) treatment, its prognosis is unfavourable in many cases. This is why many analyses of factors able to positively influence disease evolution and prognosis have been performed and it is believed their earliest detection after the actual onset of the disease allows for the appropriate therapy to be administered. Molecular immunology studies have shown that in classic Hodgkin lymphoma HRS (Hodgkin Reed Sternberg) cells derive from the germinal centre in the B cells with immunoglobulin genes rearrangement, but without B cell receptor surface expression. BCL-2 has been identified as the first gene involved in HRS cells apoptosis. BCL-2 expression by HRS cells may prevent apoptosis induced by the absence of B cells functional receptors, which explains tumorigenesis. BCL-2 expression may also explain the resistance to treatment-induced HRS cells apoptosis.

Aim: This study aims to determine BCL-2 expression in Hodgkin's lymphoma patients included in the study, correlation of its expression with biochemical and immunological changes, as well as evaluation of survival in these patients.

Methodology: A retrospective analysis has been conducted from May 2008 to April 2013 for 151 patients diagnosed with Hodgkin lymphoma in the Hematology Department, Timisoara. The main diagnostic method was biopsy, followed by histopathological and immunohistochemical examinations of harvested tissue.

Results: Mean age of patients included in the study is 49.69±17.46, with a minimum of 18 and a maximum of 89 years, out of which 37.7% were women and 62.3% men. The follow up period since diagnosis was 13.92±6.24 months up to complete remission (33.7% of patients); partial remission was seen in 45.7% of patients, 17.2% had progressive disease, 0.6% relapsed and 2.5% died. BCL-2 was detected in 82 patients (54.3%), the intensity varying according to the histological degree of the disease, as follows: BCL-2 expression is seen in 19.8% of MC patients, 39.1% of NS patients and 5.3% of patients with lymphocyte depletion.

Survival is lower in patients with BCL-2 expression than in those not expressing the gene, and gene expression shows positive, statistically significant correlation with increased levels of other immunological (CD15, CD20, CD30) and biochemical (fibrinogen) markers.

Conclusions: A reduced survival rate of patients with BCL-2 expression associated with intense expression of CD15, CD20 and CD30 markers, as well as with increased fibrinogen levels were seen in this study. Biochemical markers ESR, LDH and ceruloplasmin are inversely correlated with presence of BCL-2, and survival rates are not significantly changing at increased levels compared to normal.

INTRODUCTION

Although important progresses have been made in Hodgkin's disease (HD) treatment, its prognosis is unfavourable in many cases. This is why many analyses of factors able to positively influence disease evolution and prognosis have been performed and it is believed their earliest detection after the actual onset of the disease allows for the appropriate therapy to be administered (1).

Changes in disease activity tests (ESR, fibrinogenemia, LDH and serum alkaline phosphatase) positively correlate with a more aggressive form of disease and therefore with a more reduced rate of complete remission and shorter survival (5-year survival rate); ESR levels over 80 mm/h and serum alkaline phosphatase over 200 IU/ml are highly significant prognostic risk factors (2,3). Taken separately, ESR and serum alkaline phosphatase have very close discriminative values; their combined assessment, however, proved to be one of the most important prognostic factors, always considered in multifactorial studies and influencing HD patients survival (4-7). It is worth mentioning that increases in ESR and serum alkaline phosphatase levels should be judged in close connection with other disease activity factors. Similarly, their increased levels mirror biologically aggressive forms or presence of HD not detectable by current diagnostic procedures, as the period of complete remission is longer than 36 months in less than 30-40% of cases with these modified activity tests (8-10); thus, they might be a clue to applying since the very beginning more aggressive therapeutic protocols, such as bone marrow transplantation (11). Recently, high levels of serum TNF and its soluble receptors (p and p75) were shown to be negative prognostic factors, indicating an unfavourable evolution of HD patients (12-14).

Molecular immunology studies have shown that in classic Hodgkin lymphoma HRS (Hodgkin Reed Sternberg) cells derive from the germinal centre in the B cells with immunoglobulin genes rearrangement, but without B cell receptor surface expression (15). These changes have been attributed to defects in gene transcription with the immunoglobulin genes rearrangement in HRS cells. Some authors consider the lack of B cell receptors expression leads to apoptosis in the normal cells of the germinal centre. BCL-2 has been identified as

Received 16th of March 2014. Accepted 20th of May 2014. Address for correspondence: Potre Ovidiu, MD, PhD student, "Victor Babes" University of Medicine and Pharmacy Timisoara, Eftimie Murgu Square No. 2A, RO-300041, Timisoara, Romania, phone/fax: +40256220479; e-mail: scr-stin@umft.ro.

the first gene involved in HRS cells apoptosis. Mechanism of BCL2 involvement is partly understood. BCL-2 expression by HRS cells may prevent apoptosis induced by the absence of B cells functional receptors, which explains tumorigenesis. BCL-2 expression may also explain the resistance to treatment-induced HRS cells apoptosis (15).

AIM

This study aims to determine BCL-2 expression in Hodgkin's lymphoma patients included in the study, correlation of its expression with biochemical and immunological changes, as well as evaluation of survival in these patients.

METHODOLOGY

A retrospective analysis has been conducted from May 2008 to April 2013 for 151 patients diagnosed with Hodgkin lymphoma in the Hematology Department, Timisoara. The main diagnostic method was biopsy, followed by histopathological and immunohistochemical examinations of harvested tissue. The stage of disease was established by the means of computed tomography (CT). Polychemotherapy and number of cycles were decided based on the disease histological stage and grading. Patients' data regarding medical history and laboratory tests performed have been extracted from each patient's medical record.
Data were collected in a database and processed by the means of SPSS 17 programme. Survival rates were calculated using Kaplan-Meyer curves and correlations were established between BCL-2 presence and changes in the other biochemical parameters.

RESULTS

Mean age of patients included in the study is 49.69 ± 17.46, with a minimum of 18 and a maximum of 89 years, out of which 37.7% were women and 62.3% men. The follow up period since diagnosis was 13.92 ± 6.24 months up to complete remission (33.7% of patients); partial remission was seen in 45.7% of patients, 17.2% had progressive disease, 0.6% relapsed and 2.5% died.

BCL-2 was detected in 82 patients (54.3%), the intensity varying according to the histological degree of the disease, as follows: BCL-2 expression is seen in 19.8% of MC patients, 39.1% of NS patients and 5.3% of patients with lymphocyte depletion.

Table I. BCL-2 expression according to clinical and biological parameters

Parameter	Present BCL-2 expression	Absent BCL-2 expression
Age (mean)	54.3%	45.7%
Male gender	33.2%	29.1%
ESR (>10)	24.5%	39.7%
Ceruloplasmin (increased level)	0.7%	28.5%
LDH (increased level)	11.9%	27.2%
Fibrinogen (increased level)	30.4%	4%
CD15	54.3%	45.7%
CD20	54.3%	45.7%
CD30	54.3%	45.7%

There is an inverse, but statistically significant correlation ($p<0.01$) between BCL-2 expression and ESR, ceruloplasmin and LDH. Fibrinogen, CD15, CD20 and CD30 exhibit positive, statistically significant correlation with BCL-2 ($p<0.01$).

Table II. Correlations between BCL-2 expression and the other evaluated biochemical and immunological parameters

Parameter	Correlation	p
ESR	-0.277	<0.01
Ceruloplasmin	-0.473	<0.01
LDH	-0.447	<0.01
Fibrinogen	0.323	<0.01
CD15	0.843	<0.01
CD20	0.944	<0.01
CD30	0.866	<0.01

Survival rates in patients with present BCL-2 expression is lower in those with more intense gene expression ($p<0.01$), which is also true for CD15, CD20, CD30 markers.

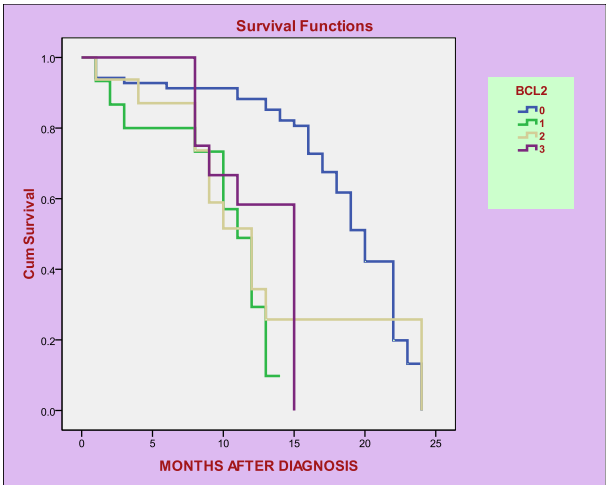

Fig. 1. Survival according to BCL-2 expression

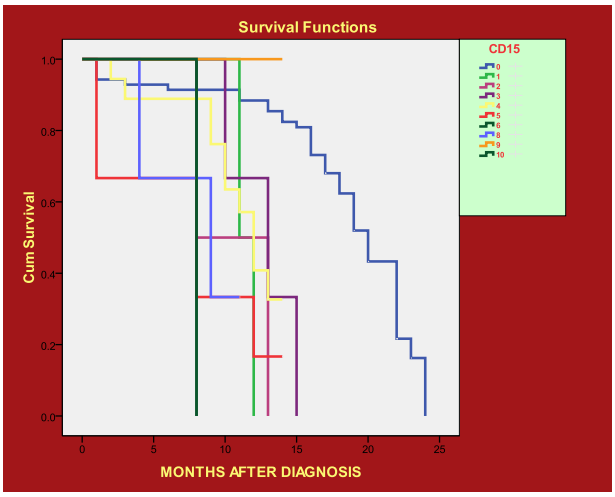

Fig. 2. Survival according to CD15 levels

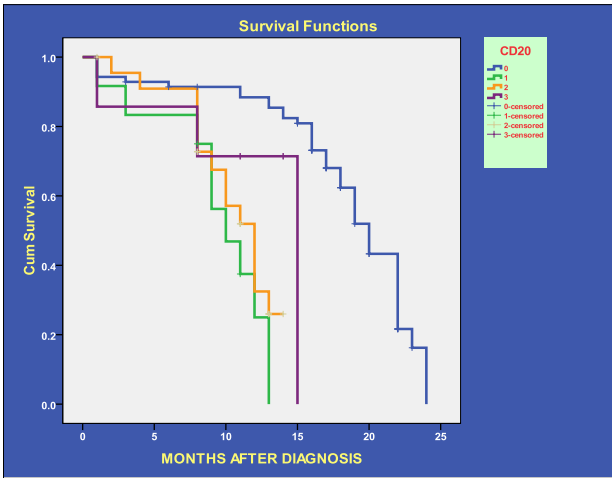

Fig. 3. Survival according to CD20 levels

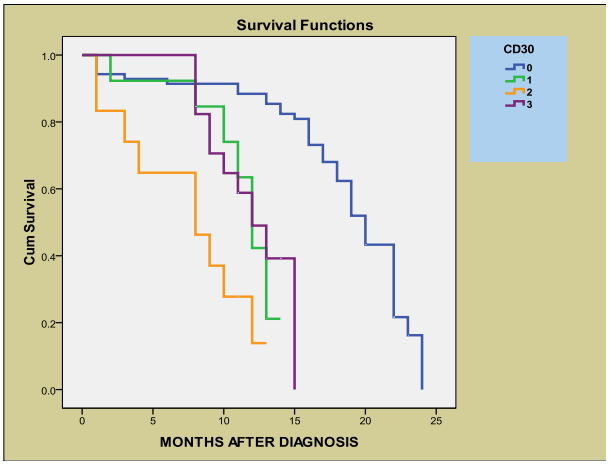

Fig. 4. Survival according to CD30 levels

Increased ESR levels are associated with survival periods equal to that of patients with normal ESR levels, being inversely correlated with BCL-2 expression, which is also seen in case of ceruloplasmin and LDH.

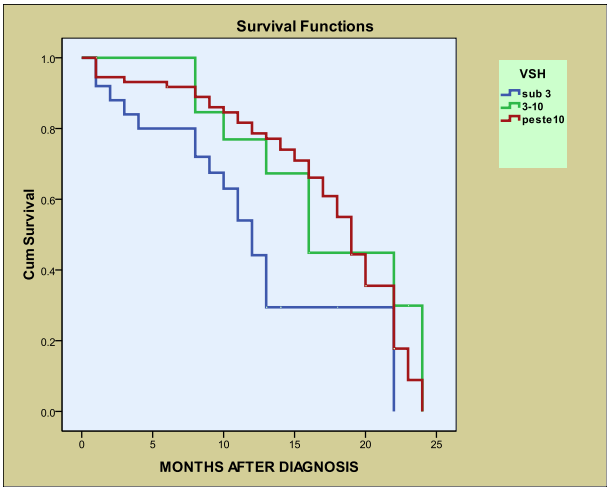

Fig. 5. Survival according to ESR levels

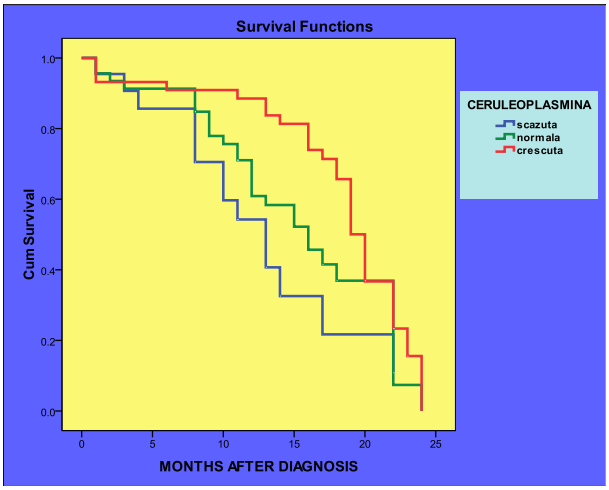

Fig. 6. Survival according to ceruloplasmin levels

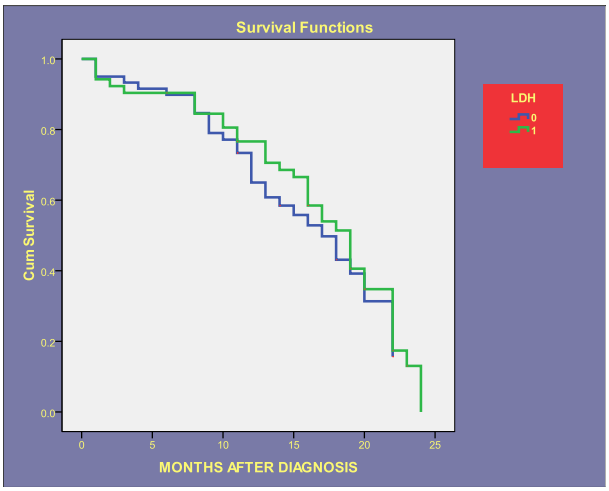

Fig. 7. Survival according to LDH levels

Conversely, increased fibrinogen levels are associated with decreased survival rates and show positive, statistically significant correlation ($p<0.01$) with BCL-2 expression.

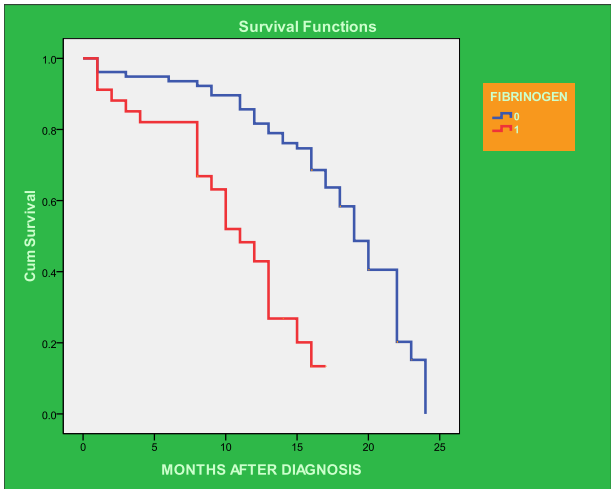

Fig. 8. Survival according to fibrinogen levels

DISCUSSION

It is shown in the literature that changes in disease activity tests (ESR, fibrinogenemia, LDH and serum alkaline phosphatase) are positively correlated with a more aggressive form of disease and therefore with a more reduced rate of complete remission and shorter survival (5-year survival rate); ESR levels over 80 mm/h and serum alkaline phosphatase over 200 IU/ml are highly significant prognostic risk factors. Our study shows reduced survival in patients with high fibrinogen levels, the survival rate being less influenced by increased ERS, ceruloplasmin and LDH levels.

Regarding the expression of immunological markers, our study shows a significant reduction in survival rates of patients with BCL-2 expression, which is closely correlated with intense expressions of the other immunological markers, namely CD15, CD20 and CD30, some authors considering that BCL-2 expression by HRS cells may prevent apoptosis induced by the absence of B cells functional receptors, which explains tumorigenesis. BCL-2 expression may also explain the resistance to treatment-induced HRS cells apoptosis.

CONCLUSION

A reduced survival rate of patients with BCL-2 expression associated with intense expression of CD15, CD20 and CD30 markers, as well as with increased fibrinogen levels were seen in this study. Biochemical markers ESR, LDH and ceruloplasmin are inversely correlated with presence of BCL-2, and survival rates are not significantly changing at increased levels compared to normal.

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EXPRESIA BCL-2 CA FACTOR DE PROGNOSTIC NEGATIV IN LIMFOMUL HODGKIN

REZUMAT

Introducere: Cu toate ca s-au facut progrese importante in ceea ce priveste tratamentul bolii Hodgkin (HD), prognosticul acesteia este nefavorabil in multe dintre cazuri. De aceea, au fost effectuate o multitudine de analize ale factorilor care ar putea influenta pozitiv evolutia si prognosticul bolii si se crede ca detectarea precoce dupa debutul bolii permite administrarea unei terapii adecvate. Studii de imunologie moleculara au aratat au aratat ca in limfomul Hodgkin clasic HRS (Hodgkin Reed Sternberg) celulele deriva din centrul germinativ al limfocitelor B cu rearanjamente genice ale imunoglobulinelor, dar fara expresia receptorilor de suprafata ai limfocitelor B. BCL-2 a fsot identificata ca prima gena implicata in apoptoza celulara a HRS. Expresia BCL-2 de catre celulele HRS poate preveni apoptoza indusa de absenta receptorilor functionali ai limfocitelor B, ceea ce explica tumorigeneza. Expresia BCL-2 poate explica de asemenea rezistenta la apoptoza celulelor HRS indusa de tratament.

Scop: Acest studiu isi propune sa determine expresia BCL-2 la pacientii cu limfom Hodgkin inclusi instudiu, corelarea expresiei acesteia cu modificarile biochimice si imunologice, precum si evaluarea supravietuirii la acesti pacienti.

Metodologie: A fost efectuata o analiza retrospectiva pentru perioada mai 2008-aprilie 2013 pe unlot de 151 de pacienti diagnosticati cu limfom Hodgkin in Departamentul de Hematologie Timisoara. Principala metoda de diagnostic a fost biopsia, urmata de examinarea histopatologica si imunohistochimica a tesutului prelevat.

Rezultate: Varsta medie a pacientilor inclusi in studiu a fost 49,69±17,46, cu varsta minima de 18 ani iar varsta maxima de 89 de ani, din care 37,7% au fost de sex feminin si 62,3% de sex masculin. Perioada de urmarire din momentul diagnosticului a fost de 13,92±6,24 luni pana la remisiunea completa (33,7% dintre pacienti); remisiune partiala a fost intalnta la 45,7% dintre pacienti, 17,2% au prezentat boala progresiva, 0,6% au avut recaderi si 2,5% au murit. BCL-2 a fost detectata la 82 de pacienti (54,3%), intensitatea expresiei fiind variabila si concordanta cu gradul histologic al bolii, dupa cum urmeaza: expresia BCL-2 a fost de 19,8% dintre pacientii cu MC, 39,1% la cei cei cu NS si 5,3% la pacientii cu depletie limfocitara. Supravietuirea a fost mai scazuta la pacientii cu expresie a BCL-2 comparativ cu cei care nu exprimau aceasta gena, iar expresia genica a aratat o corelatie pozitiva, semnificativa statistic cu niveluri crescute ale altor markeri imuni (CD15, CD20, CD30) si biochimici (fibrinogen).

Concluzii: In acest studiu am relevat o scadere a ratei de supravietuire la pacientii cu expresie a BCL-2, asociata cu cresterea expresiei markerilor CD15, CD20 si CD30, precum si cu cresterea nivelului fibrinogenului. Markerii biochimici VSH, LDH si ceruloplamina sunt corelati invers proportional cu prezenta BCL-2, iar rata de supravietuire nu este modificata semnificativ de niveluri crescute comparativ cu normalul.

OTITIS MEDIA WITH EFFUSION IN CHILDREN – A CLINICAL TRIAL

KARINA MARIN, ROXANA VINTILA, ROXANA POPESCU, ALIN HORIA MARIN, PRODEA MIHAELA, MARIOARA POENARU
"Victor Babes" University of medicine and Pharmacy Timisoara

ABSTRACT

Otitis media with effusion (OME), or 'glue ear', is very common in children, especially between the ages of one and three years following Eustachia tube impairment. OME is defined as middle ear effusion without signs or symptoms of an acute infection. OME may occur as a primary disorder or as a sequel to acute otitismedia. The functional effect of OME is a conductive hearing loss of about 25 to 30 dB associated with fluid in the middle ear.

Materials and methods: In total 250 patients with otitis media with effusion were enrolled in this study. They were diagnosed with OME between 2011- 2014 in Bega ENT Clinic Timisoara. Diagnosis was established by otomicroscopy. In children with allergic symptoms prick tests were performed. All children underwent pure tone audiometry, tympanometry, acoustic reflex. All children underwent nasal bacterial exam, nasal endoscopy. Treatment was conservative or surgical.

Results: 214 (85.6%) patients presented transmission hearing loss, with type B tympanogramm. Transmission hearing loss ranged between 25- 40 dB. 24 (9.6%) patients had type C tympanogramm. All patients had absence of ipsilateral acoustic reflex. Nasal bacterial exam showed following results: *S pneumoniae* was found in 35% of cases, *H influenzae* is found in 20%, *M catarrhalis* was present in 1% of the cases. Tympanocentesis was performed in 156 cases. Typmanotomy and adenoidectomy was performed in 176 children. Tympanotomy, adenoidectomy and ventilation tube insertion was performed in 20 cases. Complications were otorrhea, tympanosclerosis and perforation.

Key words: otitis media with effusion, transmission hearing loss, ventilation tube, adenoidectomy

INTRODUCTION

Otitis media with effusion (OME) is an inflammatory process within the middle ear space consisting of fluid accumulation. Otitis media with effusion (OME) nearly always follows acute otitis media (AOM) as it resolves. It is due to a failure of the Eustachian tube to ventilate, clear and drain secretions. Ligation of the eustachian tube in animals invariably leads to the formation of a persistent middle ear effusion. Middle ear effusions (MEE) result from inflammation within the middle ear cleft (otitis media). Non-inflammatory middle ear fluid can result from barotrauma, head trauma or a cerebrospinal fluid leak. Symptoms of acute otitis media AOM are congestion of the tympanic membrane, bulging of the tympanic membrane, fever, pain. It can lead to purulent secretions.

The newer models describe the primary event as inflammation of the middle ear mucosa caused by a reaction to bacteria already present in the middle ear. Indeed, Bluestone and others have shown (using radiographic evidence) that reflux up the eustachian tube is demonstrable in children prone to otitis media (1). Furthermore, Crapko et al demonstrated the presence of pepsin in the middle ear space of 60% of children with otitis media with effusion (2). Many factors have been implicated in the failure of the clearance mechanism, including ciliary dysfunction; mucosal edema; hyperviscosity of the effusion; and, possibly, an unfavorable pressure gradient. Otitis media with effusion is ubiquitous in children who have a cleft palate. The cause is simply the lack of proper insertion of the tensor velipalatini muscle in the soft palate. The muscle is, therefore, unable to open the eustachian tube on swallowing or wide mouth opening.

There is also a possibility that allergy can contribute to otitis media with effusion. Major classes of immunoglobulins, complement, and immune complexes of antigen and antibody are found in MEE with their concentrations being higher in mucoid effusion as compared to serous effusions.

These mediators are hypothesized to play role in the pathology of otitis media.

Mucocilliary dysfunction caused by allergy and impaired Eustachian tube function can lead to negative pressure in the middle ear resulting in transudation of liquid from the mucosa. Persistent middle ear fluid from OME leads to mobility impairment of the tympanic membrane and serves as a barrier to sound conduction.

When conservative treatment has failed, ventilation tubes are generally used for treatment and prevention of recurrence.

OME appears usually in children occurring up to 90% by the age of 2. Refractory OME can lead to hearing loss, late language acquisition, behavioral and cognitive difficulties. The most common bacteria in acute otitis media, in order of frequency, are *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis*

Received 12th of March 2014. Accepted 15th of May 2014. Address for correspondence: Karina Marin, MD, PhD Student Department of ENT, University of Medicine and Pharmacy "Victor Babeș" Timișoara, Eftimie Murgu Square No. 2A, Timisoara, RO-300041, Phone: +40723316869, e-mail: marinkrn@yahoo.com

MATERIAL AND METHODS

In total 250 patients with otitis media with effusion were enrolled in this study. They were diagnosed with OME between 2011- 2014 in Bega ENT Clinic Timisoara.

In order to establish the diagnosis and therapy all children were examined by otomicroscopy, and a complete ENT consult. Children who presented a positive family history or symptoms for allergy were submitted to allergy tests and measurement of total and specific IgE level. Skin tests were performed for perennial and seasonal allergens. Children underwent skin prick test for respiratory allergens like dust mite, pollen, mixture of herbs, molds, cat and dog epithelium. Results were evaluated after 10 minutes. Wheals greater than 3 mm at the side of the negative control were considered positive.

All children underwent pure tone audiometry, tympanometry and ipsilateral acoustic reflex.

Tympanograms were divided into the following types: type A (+ 99 to – 99 mmH_2O), type C1 (-100 to -199 mmH_2O, type C2 (more than 200 mmH_2O).

All children underwent nasal bacterial exam.

All children underwent nasal endoscopy. Children were treated conservatory with nasal corticosteroids and surgical (tympanotomy, tympantotomy and adenoidectomy, tympanotomy, adenoidectomy and ventilation tube insertion). Follow up was after 2 weeks and after 3 months after.

RESULTS

146 (58.4%) children presented with positive allergic tests. The presenting complaints were hearing loss in 196 (78.4%) patients, lack of attention in 50 patients (20%), otalgia in 187 (74.8%) patients, fullness in 200 (80%) patients, and tinnitus in 28 (11.2%) patients.

Children presented retracted tympanic membrane, fluid level, bubbles, hypervascularity.

The age of the children ranged from 4 years and 10 years with a mean age of 7.

154 (61.6%) were boys and 96 (38.4%) were girls.

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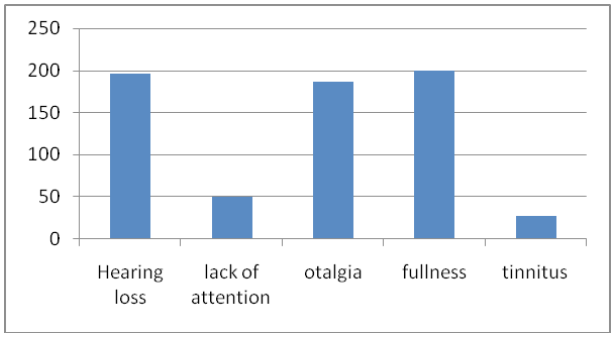

Fig. 1. Complaints presented by the patients

214 (85.6%) patients presented transmission hearing loss, with type B tympanogramm. Transmission hearing loss ranged between 25- 40 dB.

24 (9.6%) patients had type C tympanogramm. All patients had absence of ipsilateral acoustic reflex.

Nasal bacterial exam showed following results: *S pneumoniae* was found in 35% of cases, *H influenzae* is found in 20%, *M catarrhalis* was present in 1% of the cases.

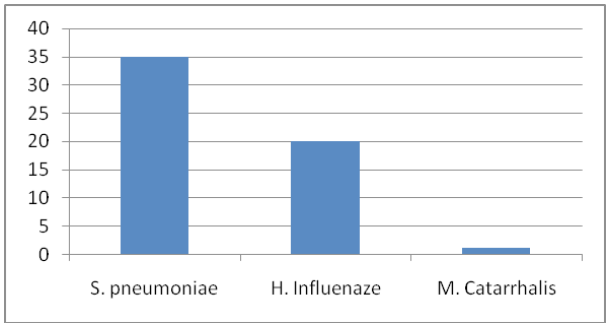

Fig. 2. Results of nasal bacterial exam

Of children younger than 2 years, 17% had recurrent disease.

This disease is sometimes associated with delayed language development in children younger than 10 years, and the loss is usually conductive, with an average air conduction threshold of 27.5 decibels (dB), but otitis media with effusion has also been associated with sensorineural hearing loss. Delayed language development was discovered in 15 cases.

Additional findings were turbinate enlargement especially in cases of allergy (52%), postnasal drip, rhinorrhea, environmental allergies.

48 patients presented allergies for dust mites, 20 patients were positive for pollen, 15 patients were positive for mixture of herbs, 15 patients were positive for molds,10 patients were positive for cats and dog epithelia.

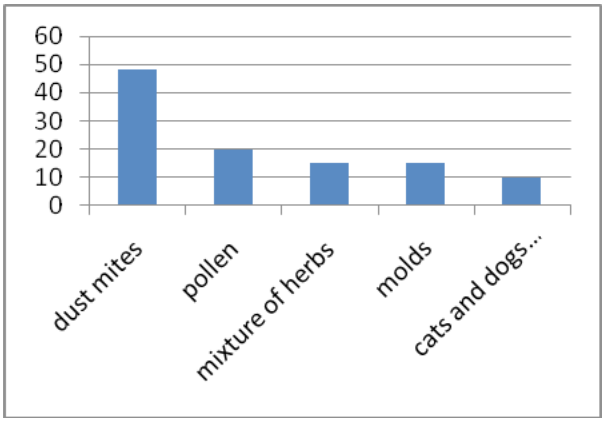

Fig. 3. Allergies presented by the patients

In general, the prognosis for otitis media with effusion was good. Most episodes spontaneously resolved without intervention, and many resolved undiagnosed.

Following spontaneous tube extrusion, 20-50% of patients will have a recurrence of otitis media with effusion, potentially requiring the replacement of pressure equalization tubes (PETs) and, in most cases, simultaneous adenoidectomy.

TREATMENT

Tympanocentesis was performed in 156 children serving both as therapeutic procedure and a diagnostic procedure. Typmanotomy and adenoidectomy was performed in 176 children, and was performed in cases of chronic nasal obstruction complicated with mucoid otitis media persisting beyond 3 months. Tympanotomy, adenoidectomy and ventilation tube insertion was performed in 20 cases asscoiated with persistent mucous otitis media or repeated otitis media with effusion.

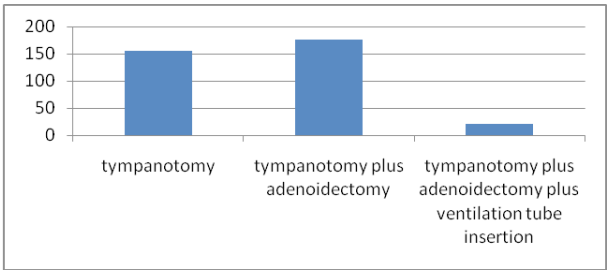

Fig. 4. Patients'treatment

In 10 children the ventilation tube was eliminated after 2 or 3 months. Most cases of effusion resolved spontaneously. Ongoing monitoring is mandatory, because the frequency of structural damage increases with effusion duration.

The overall complication rate after placement of pressure equalization tubes appeared in 7 patients. Persistent otorrhea is the most common complication, occurring in 2 patients. Second was tympanosclerosis occurring in 6 patients, which was not clinically significant unless it was extensive.

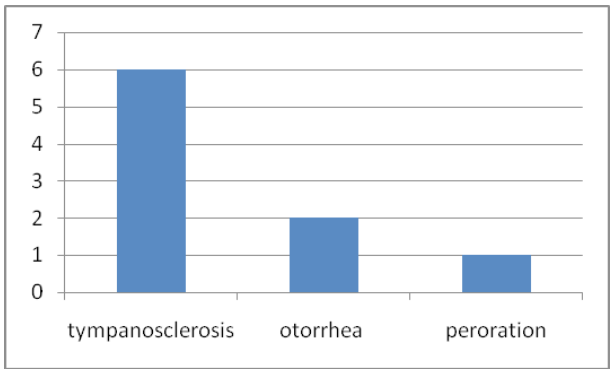

Fig. 5. Complications appearing in patients

Persistent perforation appeared in 1 patient.

Complication after adenoidectomy was bleeding which occurred in 2 patients, temporary velopharyngeal insufficiency in 4 patients. Follow up was performed after, at 2 weeks and then 3 months after. Grommets were removed after 1 year or 2 years.

DISCUSSIONS

OME is defined as a fluid in the middle ear. It is very common in children leading to hearing loss or surgical intervention. OME rate has to peaks: one around 2 years of age, and the otherat 5 years (Zielhui *et al.*). As for gender, some studies revealed that OME appeared more frequent in boy than girls. The different prevalence rate can depend also on the high atopic history. Approximately 20- 30% of the general population is atopic and 15 % of children. Caffarelli *et al*. compared 192 children with OME with a control group of 200 children in order to determine the prevalence of allergy. There were no significant differences between the 2 groups regarding the family history of allergy and skin tests. Although children with OME symptoms la rhinitis, asthma, conjunctivitis, were more frequent.

van den Aardweg *et al.* (5) saw a significant benefit of adenoidectomy as far as the resolution of middle ear effusion in children with OME is concerned. However, the benefit to hearing is small and the effects on changes in the tympanic membrane are unknown.

Lous *et al.* (4) indicates that the benefits of grommets in children appear small. The effect of grommets on hearing diminished during the first year. Potentially adverse effects on the tympanic membrane are common after grommet insertion. Therefore an initial period of watchful waiting seems to be an appropriate management strategy for most children with OME.

Boonacker *et al.* (3) found that adenoidectomy is most beneficial in children with persistent OME aged ≥ 4 years. A smaller beneficial effect was found in children with recurrent AOM aged < 2 years. Browning (2) explained that in children with OME the effect of grommets on hearing, as measured by standard tests, appears small and diminishes after six to nine months by which time natural resolution also leads to improved hearing in the non-surgically treated children.

Numerous systematic reviews have confirmed the efficacy of pneumococcal vaccine in preventing pneumococcal AOM. Following introduction of the 7-valent pneumococcal conjugate vaccine (PCV7), childhood incidence of AOM declined in the USA and Canada. In children aged under 2 years, a 43% reduction in AOM.

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OTITA MEDIE SEROASA LA COPII – STUDIU CLINIC

REZUMAT

Otita medie seroasa apare in special la copii cu varste cuprinse intre 1-3 ani ca urmare a unei disfunctii a tubei lui Eustachio. Transudatul poate apare de prima intentie sau urmand unei infectii acute. Este insotita de hipoacuzie de transmisie de 25- 30 dB.

Material si metoda: In studiu au fost inclusi 250 de pacienti. Acestia au fost diagnosticati cu otita seroasa pe perioada 2011- 2014 in Clinica ORL Bega Timisoara. Diagnosticul a fost stabilit ptin otomicroscopie. In cazul copiilor cu simptome alergice s-au efectuat prick teste. Toti copiii au fost supusi audiometriei tonale, timpanometriei, reflexului stapedian, endoscopiei nazale. Tratamentul a fost conservator sau chirurgical.

Rezultate: 214 (85,6%) din pacienti au prezentat hipoacuzie de transmisie intre 25- 40 dB, cu timpanograma de tip B, 24 pacienti (9,6%) au prezentat timpanograma tip C. In cazul tuturor pacientilor reflexul stapedian a fost abolit. Exudatul nazal a relevant urmatoarele: *S pneumonia* a fost pozitiv in 35% din cazuri, *H influenza* in 20% din cazuri, *M catarrhalis* in 1% din cazuri. In 156 de cazuri s-a efectuat timpanotomie. Timpanotomie plus adenoidectomie s-a efectuat la 176 de cazuri. Adenoidectomie si insertie de tub de dren s-a efectuat in 20 de cazuri. Complicatiile aparute au fost otoreea, timpanoscleroza si perforatia.

Cuvinte cheie: otita media seroasa, hipoacuzie de transmisie, tub de ventilatie, adenoidectomie

MULTIPLE MYELOMA AND STENT RESTENOSIS IN OBSTRUCTIVE CORONARY ARTERY DISEASE

VALCOVICI M, PASCALAU L, IANCU O
"Victor Babes" University of medicine and Pharmacy Timisoara

ABSTRACT

The cardiotoxicity of chemotherapeutics is a reality. The association between cardiovascular risk factors and coronary events risk, during the chemotherapy may precipitate an acute coronary syndrome that requires a fast approach to myocardial ischemia. We present the case of a multiple myeloma patient who 2 months after the initiation of chemotherapy treatment was associated with an acute coronary syndrome which required interventional revascularization. The appearance of restenosis at the resumption of chemotherapeutic cure raises the problem of pathophysiological interpretation of the proinflammatory phenomenon.
Keywords: multiple myeloma, anthracyclines, acute coronary syndrom, restenosis.

INTRODUCTION

Cardiotoxicity is defined by the disfunction of parameters which alters the global systolic function, as well as the electrophysical parameters of the heart during the treatment with chemotherapeutic agents dose-dependent.

Cardiotoxicity induced by chemotherapeutic agents, for example anthracyclines, is considered to be resistant to conventional treatments. For many of these patients, the heart disease mortality risk will become equal or even higher than the malignancy recurrence risk. Certain forms of **chemotherapy** have been directly associated with **cardiotoxicity.**

Current guidelines recommend that during the treatment with chemotherapeutic agents to evaluate the systolic function through different imagistic techniques. The cardiotoxicity consequences may interest the coronary vasomotricity in presence of traditional risk factors. In some cases, interventional or surgical revascularization of these patients is the standard and obligatory procedure. For those interventional revascularized through endovascular prosthesis placement, the restenosis remains the major complication of these procedures.

We present the case of a patient with multiple myeloma history who develops after about 2 months after the initiation of the chemotherapeutic agents treatment (see 2 applications: vincristine 0.4 mg continuous perfusion, anthracycline 9 mg / m² continuous perfusion, dexamethasone 40 mg, on day 1-4) in the first month, 1 month break then initiates the administration of velcade 1.3 mg / m ² (days 1-4, and 8-11), develops an episode of acute coronary typical pain.

CLINICAL CASE

The patient, S.A., aged 67 years, with cardiovascular risk factors presents on one side hemodynamic: average aortic stenosis and arterial essential hypertension stage II, metabolic: LDL c (167 mg / dl), ischemic through STEMI and through stenosis on LAD, multiplied risk through chronic renal failure stage III, with multiple myeloma IgG kappa III B, shows up with costrictive retrosternal intense pain which succumbs to nitroglycerin, being clinically, electrically and biologically diagnosed with previous acute myocardial infarction with ST segment elevation.

The ECG can detect 1-2 mm ST segment elevation in V1-V2 and depression 2 mm in V4-V6 (Figure 1). The transthoracic ecocardiography reveals normal size VS, with concentric left ventricular hypertrophy, global systolic function slightly altered (ejection fraction of 48%), global hypokinesis, degenerative changes of mitral aortic apparatus, mitral regurgitation level III, average aortic stenosis, dilatation of the left atrium. It is decided the performing of the coronary angiography that can detect: left dominant coronary system, aortic and coronary calcifications visible through fluoroscopy. ACS, ACX, ACD trunk without significant damage. Stenosis 80% - 85%, short with inhomogeneous appearance average LAD, distal vessel without angiographic significant lesions (Figure 2). From the stenosis zone emerges Diagonal I, which presents an ostial stenosis of 30%, distal vessel without significant lesions. It was conducted the interventional permeabilization to average level LAD (according to secondary prevention guidelines of the obstructive cardiovascular disease), practicing the direct stenting of the lesion from average level LAD with BMS Integryti 3.0 / 18 mm stentys, with good final result, without residual stenosis, without proximal or distal dissection (Figure 3).

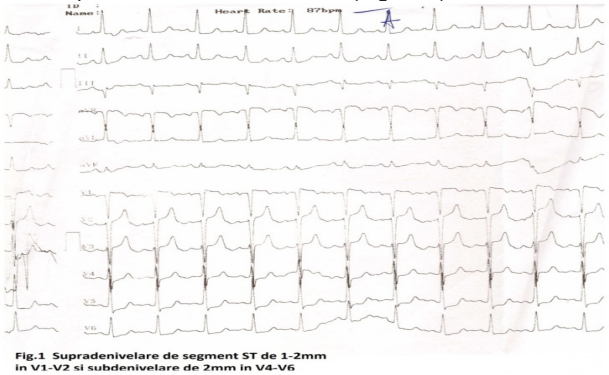

Fig. 1. 1-2 mm elevation of ST segment in V1-V2 and 2 mm depression in V4-V6

Received 13th of March 2014. Accepted 14th of May 2014. Address for correspondence: Valcovici M, MD, PhD Student Department of Cardiology, University of Medicine and Pharmacy "Victor Babeș" Timișoara, Eftimie Murgu Square No. 2A, Timisoara, RO-300041, Phone: +40740250950, e-mail: mihaeladanielacardio@gmail.com

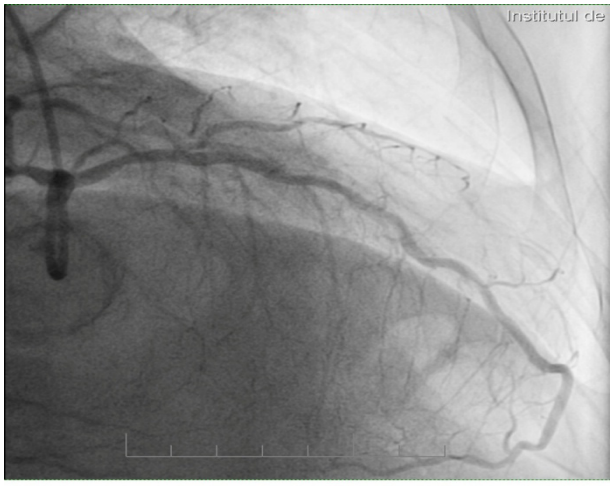

Fig. 2. Coronary vessel re-permeabilization

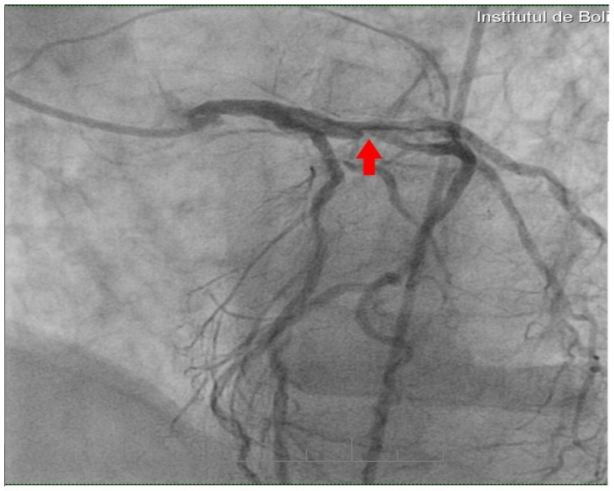

Fig. 3. Intrastent stenosis

The patient is discharged stable, hemodynamically, asymptomatic, with the following recommendations: regular cardiological evaluation, including ultrasound monitoring, the continuing of the basic medical treatment associated with dual anti-aggregation platelet. In about 4 months from the coronary injury, the patient shows up in a specialized hospital unit for recurrence of angina type pain accompanied by dyspnea of orthopnea nature which hardly succumbs to nitroglycerin. The EKG can detect ST-segment depression of 2-3 mm in V4-V6 (Figure 1). The performance of a new angiographic exploration highlights intrasent restenosis, with 90% lumen reduction to the average LAD (Figure 4). Initially, it is performed balloon angioplasty with good angiographic final result. The post-procedural 24 hours, the patient accuses retrosternal pain of constrictive nature, with changes the dynamics of the electrical rest circuit path, ischemic-lesional type changes in the previous territory. It is resumed the angiography procedure and it is performed direct stenting with pharmacological stent active Xience 2.75 / 38 mm, at average level LAD with good final result, without proximal or distal dissection (Figure 5).

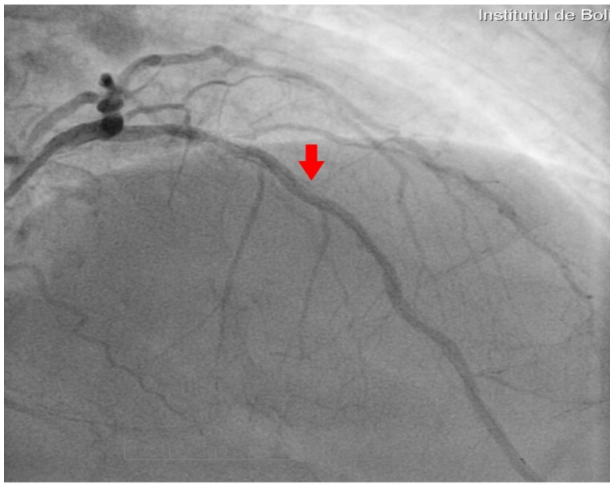

Fig. 4. Interventional procedure at the level of LAD

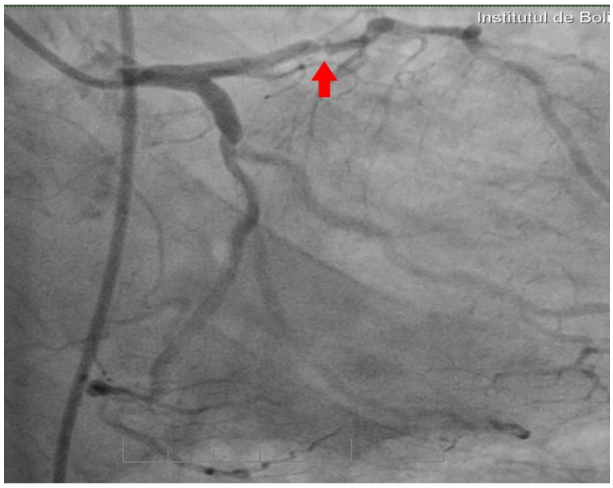

Fig. 5. 80% lesion at the level of LAD/calcified LAD

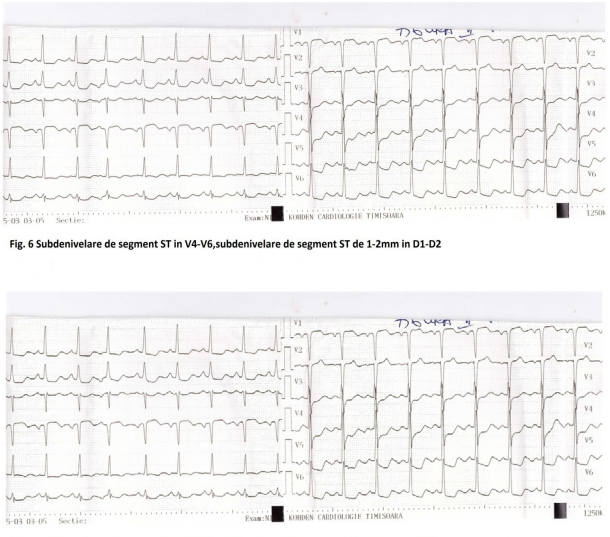

Fig. 6. ST segment depression in V4-V6, 1-2 mm depression of ST segment in L1-L2

DISCUSSION

The presented case illustrates on one side the additional risk of chemotherapeutic cardiotoxicity with anthracycline in presence of a high cardiovascular risk, multiplied by the presence of chronic kidney disease stage III. (RFG 38.94). Therefore, ischemic risk through the angiocoronarography evaluation in presence of the metabolic risk through LDLs increased.

The conducted studies to this date, demostrate the direct association relationship between cardiovascular risk factors and anthracycline cardiotoxicity (guideline ACC / AHA published in JACC in 2014), reveals the possibility of early cardiovascular complications and insists on the prediction strategies of subsequent cardiotoxicity at the initiation of the treatment with anthracyclines.

The peculiarity of the case focuses on the intrastent restenosis phenomenon, which is well-known complication after the revascularization procedure through coronary endovascular prosthesis. It is clear that the inflammation plays an important role in the pathology of Pathway - proteasome restenosis, remains crucial in the controlling of the biological process that includes inflammation, cell proliferation and apoptosis, events that contributes to the neo-intima formation.

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MIELOMUL MULTIPLU SI RESTENOZA INTRASTENT IN BOALA CORONARIANA OBSTRUCTIVA

REZUMAT

Cardiotoxicitatea chimioterapicului este o realitate. Asocierea intre factorii de risc cardiovasculari si riscul evenimentelor coronariene, in cursul chimioterapiei poate precipita un sindrom coronarian acut ce necesita o abordare rapida pentru miocardul ischemic. Prezentam cazul unui bolnav cu mielom multiplu care la 2 luni dupa initierea tratamentului chimioterapeutic, s-a asociat cu un sindrom coronarian acut ce a necesitat revascularizare interventionala. Aparitia resetenozei la reluarea curei chimioterapeutice ridica problema de interpretarea fiziopatologica a fenomenului proinflamatoar.

Cuvinte cheie: mielom multiplu, antracicline, sindrom coronarian acut, restenoza.