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Present interest in ocular toxoplasmosis treatment

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Although antitoxoplasmic treatments were marketed 50 years ago and are only active on the forms that are rapidly multiplying, they remain in use all over the world. The commonly used antitoxoplasmic drugs are Sulfadiazine and Pyrimethamine, but also, Sulfamethoxazole—Trimethoprim, Atovaquone, Spiramycin, Clindamycin. No drug is really efficient on the cysts, they will usually not eradicate infection. Atovaquone was investigated briefly as a possible cysticidal agent. Treatment for ocular lesion are described. The location of the lesion in relation to the macula and optic disc, and the size and degree of vitritis induced by the lesion, are the main factors regarding the decision to start therapy. In the presence of a proeminent anterior segment inflammation, we have a low threshold for the initiation of topical steroids. The last research are in direction of strategy for vaccine development.
Key words: toxoplasma gondii, ocular lesion, antimicrobian agent

The commonly used antitoxoplasmic drugs, sulfadiazine and pyrimethamine are active in vitro against Toxoplasma gondii tachyzoites. The cure performed for 4 to 6 weeks is the most active in acute, reccured and congenital toxoplasmosis. Although, this treatment were marketed 50 years ago and are only active on the forms that are rapidly multiplying, is remain in use all over the world. No drug is really efficient on the cysts. Atovaquone was investigated briefly as a possible cysticidal agent.

Pyrimethamine: initial dose for adult is 200 mg, after this 50 – 75 mg/day. For newborn children with congenital disease 0,5 – 1 mg/day oral or parenteral.

Sulfadiazine: dose for adult is 4-6 g in 4 administration/day. For newborn children with congenital disease 100 mg/day for 1 year.

Although this medications are indicated in acute disease, when the replications of the parasite is intense, are not able to eradicate the infection. Is believed this drugs have low effects in subclinic disease, although they reduced the cysts dimensions. Others medications, as diaminophenilsulfone, Atovaquone, Spiramycin, Clindamycin are indicated in difficult cases.

For pregnant woman spiramicine is indicated 2- 3 g/day (4 doses per oral), cure of 3 weeks within 2 weeks pause. This is the least toxic antibiotic used in toxoplasmosis. In the first three months, therapeutic interruptions of pregnancy is recommended. Mother could be treat, the risc is reduced in that period. Spiramycin does not cross blood-ocular barriers, so indication for pregnant woman is only to sterilized the parasite passing the placenta. If the foetus disease is produced, his treatment with pyrimidine and sulphadiazine is recommended. Mother treatment will perform only in visual lost, her treatment could be dangerous for foetus.[13]

Pyrimidine, sulphanilamide, sulfamethoxazole—trimethoprim, prednisone are considered group C of securety for pregnancy. Tetracicline are group D, unsafe.

Sulphonamides are contraindicated in the first 2 quarter of pregnancy. Atovaquone, Spiramycin, Clindamycin, Azytromicine are safe and effective. The risk of teratogenity is not excluded. Intraocular therapy less teratogen with clindamycine and dexamethasone is considered.

A sistemic therapy for necrotising retinochoroiditis: initiate systemic therapy for both ocular toxoplasmosis (trimethoprim/sulfamethoxazole—trimethoprim, 160 mg, sulfamethoxazole, 800 mg; twice daily) and viral retinitis (valganciclovir 900 mg orally twice daily) while waiting for the results of the PCR testing to return. If the PCR is positive for toxoplasmosis and negative for herpes viruses, continue the trimethoprim/sulfamethoxazole for 4 to 6 weeks.

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Others preferred initial drug sulfadiazine 1 g orally four times daily and pyrimethamine 75 mg orally daily. If there is an allergy or intolerance to pyrimethamine, clindamycin 300 mg orally four times daily, or atovaquone 750 mg three times daily supplemented with folinic acid 5 mg every other day. For prophylaxis of recurrence of ocular toxoplasmosis, trimethoprim/ sulfamethoxazole has been proven to be effective.

Pyrimethamine could be given as 75 mg loading dose on day 1 and then 25 mg/day. Pyrimethamine is discontinued after 1 week. In long treatment determine bone marrow suppression. Clindamycin is given as 300 mg q.i.d. The sulfadiazine and clindamycin are continued for 3 weeks and then usually stopped. For immunocompromised patiens, treatment for 6 weeks is indicated. For reccurent infection, especially in immunocompromised AIDS, transplants host trimethoprim/sulfamethoxazole 1 daily for 1 year to 20 months has been proven to be effective.[12] Macula lession could determine recurrence. But alergic dermatitis to this drug indicate to replace it with one of this: clindamycin, tetracyclin, doxicyclin, azytromicin, atovaquone. For other authors, this medications as non-cysticidal could not be indicate for prevention.

In ocular lesions with no visual loss is indicate minocycline 100 mg daily or azytromicine. Minocycline is a solution for long treatment, prophylaxis of recurrence 6 months.

To avoid toxicity trimethoprim/sulfamethoxazole 1 daily 3 days/week 3 months.

In addition, each of these drugs has been associated with what might be considered serious side effects, including leucopenia in patients taking pyrimethamine, Stevens-Johnson syndrome in patients taking sulfa-based medications, and patients with infectious colitis who are taking oral clindamycin.

Pregnant women with ocular toxoplasmosis should be treated with spiramycin because it is believed that it reduces the rate with which Toxoplasma gondii infection is transmitted to the fetus.[5]

The principal indications for medical therapy include:

-Toxoplasmic retinochoroiditis in immuno-compromised patients, newborns, and pregnant women with acquired disease

-Lesion greater than one optic disc diameter

-Lesion associated with a large zone of retinal hemorrhage

-Visual loss with a two-line drop from the visual acuity before infection

-Moderate or severe vitreous haze

-Concomitant presence of multiple active lesions

-Persistence of an active peripheral lesion for more than 1 month

-Macular and peripapillary lesions or lesions associated with branch retinal vein occlusion.

The location of the lesion in relation to the macula and optic disc, and the size and degree of vitritis induced by the lesion, are the main factors regarding the decision to start therapy.[9]

Periferial lesion in normal immune host produced spontaneous healing in a few months. Could be indicate treatment for best evolution.

Patients with atypical features of retinochoroiditis, those with extensive lesions, and those resistant to specific antimicrobial drugs have to be evaluated. These conditions are frequently observed in immuno-compromised hosts, especially those with AIDS, but also in tumors, drugs, older patients.[3]

In patients with AIDS, it will happen for those patients treated with highly active anti-retroviral therapy, who demonstrate an increase of CD4 cells above 200 cells/mm^3.

For pregnant woman with immunodeficiency fulminant toxoplasmosis can occure. Pyrimethamine is necessary.

In the presence of a prominent anterior segment inflammation, we have a low threshold for the initiation of topical steroids. Systemic steroids may be started in 36 to 48 hours after the initiation of antibiotic therapy in immunocompetent patients.[11]

Prednisone is usually begun as 60 or 80 mg every other day at breakfast. Each week the prednisone dose is dropped by 20 mg, so that on week 2 they take 40 or 60 mg every other day. At week 3 or 4 the prednisone is discontinued. If there is a problem with taking prednisone, such as immunosuppression or poorly controlled diabetes mellitus, prednisone is not used. Patients treated without prednisone have slower improvement than when it is used. Others propose intravenous pulses of methylprednisolone for 3 days followed by oral prednisone (0.5 mg/kg/d) with subsequent tapering for a period of 4 to 6 weeks.

Systemic steroids are usually administered in the face of severe vitritis, active lesions located at the posterior pole, or those associated with severe papillitis and those with central or branch retinal vein occlusion.

Some authors add topical corticosteroids to treat the secondary anterior chamber reaction associated with toxoplasmic retinochoroiditis. The frequency of treatment is based on the severity of the reaction and patient's signs and symptoms.[4] Periocular injections of corticosteroids should be used with caution for special patients. More use periocular injections because intraocular injections is dangerous. Intraocular corticosteroid injections are much more effective and could be used for severe toxoplasmic ocular lesions in patients who present formal contraindication of oral or parenteral corticosteroid use and always with the association with specific antitoxoplasmic medication for more than 6 weeks.

Topical steroids are very frequently added in the presence of anterior uveitis.

Some authors consider treatment with topical corticosteroids could reduce immunity and aggravate ocular lesions. Many avoid the use of long-acting depot formulations of corticosteroids such as triamcinolone acetonide or triamcinolone diacetate for this same reason. They think the use of shorter-acting periocular or intraocular corticosteroids, such as dexamethasone,

together with an antimicrobial agent is perfectly acceptable, however. The duration of action of corticosteroids is not well known. Were previously detected non-negligible concentrations of residual triamcinolone in subTenon depots, more than 4 months after periocular injection.

Toxoplasmic retinochoroiditis may be associated with anterior uveitis, secondary glaucoma, and posterior synechiae. Topical steroids are mandatory in these cases.

Lastly, it should be remembered that the seroprevalence of anti-Toxoplasma gondii IgG antibodies is quite high in most countries, from approximately 25% in the United States to 75% or more in Central and South America, and so a sizable proportion of patients with retinitis and moderate to severe vitritis will be IgG-positive despite the fact that toxoplasmosis is not the cause of their uveitis.

Some reports non successful laser coagulation for active toxoplasmosis. There have been studies showing that this therapy does not make a difference in the rates of recurrence.[14] Laser photocoagulation for toxoplasmosis is not something that we use in our practice, but it may deserve further consideration. Clearly will not prevent all recurrences, some of which develop in areas remote from preexisting scars, presumably from tissue cysts that are present in normal-appearing retina. Laser photocoagulation is usually not used as a therapeutic tool during toxoplasmic retinochoroiditis. It doesn't have any effect on tachyzoites or tissue cysts. However, in some situations, laser photocoagulation may strengthen the retina submitted to vitreous tractions near an active or cicatricial lesion and therefore prevent further retinal detachment. Cryotherapy has not yet been studied enough.

Vitrectomy recommending to clear residual vitreous debris is taken generally for about 6 months following complete resolution of the inflammatory process, and certainly depending on the symptoms reported by the patient. In most cases the vitreous clears enough so that surgery is not required. Others found that vitrectomy is rarely necessary in patients with ocular toxoplasmosis 4-5 months after treatment. With patience, the vitreous haze and debris almost always clear after the retinal lesion becomes inactive, although it can take 2 years or longer. If the patient presents with vitreoretinal traction as well as retinal detachment, some perform the vitrectomy as soon as possible, others 2 months later.

Ocular toxoplasmosis may reactivate after cataract surgery.[1] If the patient undergoing cataract extraction does not have allergies or contraindications to antiparasitic drugs, it may be safer to treat rather then not to treat. Atovaquone is preferred drug of choice due to low frequency of side effects. They start treating the patient with it 3 days before the surgery and continue for 4 more weeks. Typically use trimethoprim/sulfamethoxazole twice daily for 1 week prior and 3 weeks postsurgery. Some treat vision-compromising reactivated lesions with systemic azithromycin. Others prescribed such secondary prophylaxis to a few high-risk patients, but only for those with a known history of recurrences, and those for whom the fovea would be at risk with additional episodes of active disease; in patients who appear to have frequent recurrences using minocycline 100 mg b.i.d. for 6 months.

Immunization with inactivated parasites or recombinant proteins has at best shown partial protection. Was constructed a plasmid expressing the SAG1 surface antigen of Toxoplasma gondii, p1tPASAG1, and showed that animals immunized with the plasmid produce anti-SAG1 antibodies which recognize the native SAG1. Mice immunized with p1tPASAG1 showed 80 to 100% protection against challenge with the non-cyst-producing, virulent RH isolate, compared to an 80% mortality in mice immunized with empty plasmid, which is the greatest efficacy of any vaccine against Toxoplasma gondii produced so far. The SAG1 molecule was analyzed for potential cytotoxic T-lymphocyte (CTL) epitopes, and four peptides with the best fit were synthesized. The ability of the peptides to stimulate γ interferon production by CD8+ T cells from p1tPASAG1-immunized mice was tested in an ELISPOT assay, and one new CTL epitope was identified. Adoptive transfer of CD8+ T cells from p1tPASAG1-immunized to native mice showed partial protection. In conclusion, DNA vaccination with p1tPASAG1 gave effective protection in mice against Toxoplasma gondii infection and the protection could be adoptively transferred by purified CD8+ T cells.[15]

To evaluate the immune responses induced by experimental DNA construct encoding Toxoplasma gondii surface antigen1 (SAG1) and rhoptry protein 1 (ROP1) in mice as a hybrid gene. Truncated SAG1 and ROP1 DNA fragments were amplified using polymerase chain reaction (PCR) and inserted into pEGFP-N3 vector to construct recombinant plasmid pSAG1-ROP1. Immunization with a liposome-encapsulated DNA construct encoding the Toxoplasma gondii SAG1 and ROP1 can induce humoral and cell-mediated immune responses.

The propagation of Toxoplasma gondii in the host is based on the premise that the parasite itself invades the cells. Both surface antigen 1 (SAG1) and rhoptry protein 1 (ROP1) play important roles in penetration of host cells, and those antibodies specific for these two antigens are capable of inhibiting host cell invasion.[2]

DNA vaccination is becoming more highly recognized as a promising measure for controlling and preventing infectious diseases. Meanwhile, in order to improve the effectiveness of the immune response and make the administration easier, novel strategies for vaccine development and delivery are being developed. One of the recent strategies that have potential to achieve the goal is polynucletide immunization, especially as a means of preventing infection by pathogens with multiple-stages life cycles.

The recombinant DNA construction used for vaccination were based on the plasmid vector pEGFP-

N3, which contained the human cytomegalovirus promoter and herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal. The pEGFP-N3 encodes enhanced green fluorescent protein (EGFP) which has been optimized for brighter fluorescence and higher expression. The genes encoding SAG1 and ROP1 were PCR amplified from genomic DNA of Toxoplasma gondii RH isolate. The generated PCR fragments were subjected to sequence analysis. The resulted construct was named pSAG1/ROP1. The orientation of the cloned genes was determined by an enzyme restriction analysis.

The mechanism of resistance against reactivation of infection with Toxoplasma gondii is strong dependent of g interferon (IFN-γ). Protective immunity against Toxoplasma gondii is known to be mediated mainly by T lymphocytes and γ interferon (IFN-γ). The contribution of $CD4^+$ and $CD8^+$ T-lymphocyte subsets to protective immune responses against Toxoplasma gondii infection. Both $CD4^+$ and $CD8^+$ T cells produced IFN-γ, and the $CD8^+$ T-cell subset had Toxoplasma gondii-specific cytolytic activity. Results suggest that although $CD4^+$ T cells are the strongest producers of IFN-γ, $CD8^+$ T cells are the major effectors of the vaccine-induced protection against acute toxoplasmosis.

Indoleamine 2,3-dioxygenase (INDO) is an enzyme that catalyzes the initial rate-limiting step of tryptophan (Trp) catabolism to N-formylkynurenine and kynurenine (Kyn). Many human cell lines express INDO upon stimulation with IFN- γ. Restriction of available Trp due to degradation by INDO leads to the control of various intracellular pathogens, including Toxoplasma gondii, in both nonprofessional phagocytic cells (NPPC) and professional phagocytic cells (PPC). In the absence of Trp, an essential amino acid for Toxoplasma gondii, parasite growth also becomes restricted. In fact, in human NPPC the induction of INDO appears to be the main mechanism by which IFN-γ controls the intracellular replication of Toxoplasma gondii tachyzoites.[11]

Dartmouth Medical School geneticists in the Department of Microbiology and Immunology have discovered how to weaken a common human parasite to prevent disease in an animal model after infection by the normal parasite.

The work, reported in 2002, opens new avenues for the development of vaccines and other treatments for diseases such as toxoplasmosis caused by a diverse, but related group of protozoan parasites. They found that inactivating a single enzyme in a key biochemical pathway prevented Toxoplasma gondii from causing disease and have devised a mutant Toxoplasma gondii strain that causes no disease and, more importantly, provides protection against the normal parasite. Because of the extraordinary ability of this mutant parasite to infect the animal host without apparent ill effects, this mutant could be used as a prototype vaccine strain. This parasite is amenable to further genetic manipulation; it has an amazing ability to elicit a strong immune response that is likely to be beneficial for certain vaccines targeted against other challenging infectious diseases or cancer.

Parasites have been around a long time and have become very proficient at stealing things from their host, if they can. This parasite seemed to rely on just one pathway for obtaining some of the key components of its genetic material. Most other organisms have retained two functional pathways: they can make components new (de novo), or they can salvage them from their nutrient environment. The investigators determined that the parasite relied on a sole pathway. They showed that knocking out a single enzyme of the de novo pathway blocked Toxoplasma gondii from making the essential pyrimidine components of parasite DNA and RNA. This in turn destroyed the parasite's ability to replicate and survive in an animal host, neutralizing its virulence.

Pyrimidines comprise two of the four DNA and RNA units (nucleobases). If the ability of the parasite to make its pyrimidines de novo is destroyed, actually grow the type of parasite mutant were seeking.

Using a novel genetic approach, knocked out the first and key regulatory enzyme, carbamoyl phosphate synthetase II, in the de novo pyrimidine pathway. Inactivating this enzyme made the parasite completely dependent on the added pyrimidine nucleobase uracil for its growth in the laboratory. These results suggest that pyrimidines are limited in the host cell, explaining why these parasites have retained the ability to synthesize these essential (genetic) precursors.[8]

Were expecting to observe a modest difference between the virulence of the mutant compared to its highly virulent parent. Instead, the mutant did not cause any disease in a mouse model.

One dose of the parental type parasite will kill a mouse. Yet millions of the new mutant parasites inoculated into mice do no harm; all mice survive without any sign of disease. More surprisingly, this mutant parasite was equally avirulent in mice with severe immune deficiency. It is the only known Toxoplasma gondii parasite strain that does not cause disease in immune-deficient animals.[9]

Building on the vaccine possibility, the researchers immunized mice with a single dose of their mutant strain. Weeks later they challenged the immunized mice with a lethal dose of a highly virulent Toxoplasma gondii parasite. The mice were completely protected from a lethal challenge infection. These mutants may offer great potential as a strategy for vaccine development.

Following invasion into the host cell, the protozoar Toxoplasma gondii secretes a variety of proteins that modify the parasitophorous vacuole. Within the vacuole, the 28-kDa dense granule protein known as GRA2 is specifically targeted to the tubulovesicular network which forms connections with the vacuolar membrane. To investigate the importance of GRA2, was derived from strain RH a mutant Toxoplasma gondii line in which GRA2 was disrupted by replacement with the marker Ble (selecting for phleomycin resistance).

The D-gra2 mutant invaded and grew normally in both fibroblasts and macrophages in vitro; however, it was less virulent during acute infection in mice. The survival rate of mice inoculated with D-gra2 was significantly higher; some infected mice survived the acute infection, whereas all mice infected with the wild-type strain RH succumbed to early death. Chronic infection by D-gra2 was detected by positive serology, immunohistochemical detection of parasites and cysts in the brain, and reisolation of parasites by bioassay at 6 weeks postinfection. Thus, absence of GRA2 partially attenuates the virulence of Toxoplasma gondii during the acute phase of infection and allows for establishment of chronic infection by the otherwise highly virulent RH strain. These results establish that GRA2 plays an important role during in vivo infection and provide a potential model for examining acute pathogenesis by Toxoplasma gondii.[8]

They are a few drugs widely used for treatment of toxoplasmosis, they will usually not eradicate infection. It is believed that these drugs have little effect on subclinical infections. The last research are in direction of strategy for vaccine development.

Because we have a lot of insuccesfull results in treatment of toxoplasmosis, especially the ocular one we have to reconsidere our efforts in preventing and early detection off this disease.

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Experimental study on mice regarding the influence of melatonin on the dinamics of different tissues lipid peroxidation

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Background. Melatonin (N-acetyl-5-methoxytryptamine) is the main hormone biosynthesized by the pineal gland. This biomolecule coordinates and synchronizes the expression of the most important physiologic sleep-awake effects and biological rhythms. Numerous studies in the scientific literature also describe its multi-regulatory roles in the endocrine, immune and nervous systems. Recently melatonin was reported to be a free radical scavenger, having a particularly interesting antioxidant mechanism.

Aim. In this regard, the aim of our study was to evaluate the protective effect (the antioxidant capacity) of melatonin over tissues lipid peroxidation.

Materials and methods. Studies were conducted on mice, divided into two working groups: the control group (mice that received water ad libitum) and the melatonin group (mice for which the drinking water was substituted by a 2 mg/L melatonin solution), that were kept under these conditions for 3 weeks. We evaluated the susceptibility to lipid peroxidation on these groups of animals using the following tissue homogenates: brain and liver.

In this regard we quantified tissues MDA (malonyl-dialdehyde) levels at the beginning of the experiment (initial time – T_0) and after 24 hours of incubation (37°C)-T_{24}. The assay is based on the reaction of MDA with thiobarbituric acid (TBA), forming a MDA-TBA2 adduct that strongly absorbs at 535 nm. MDA can be found in most biological samples as a result of lipid peroxidation, and has become one of the most widely reported analytes for the purpose of estimating oxidative stress effects on lipids.

Results and discussion. Our experiments bring new data regarding melatonin's protective effects as antioxidant. The efficacy of this biomolecule proved to be at the highest level in the case of both cerebral and hepatic level.

***Key words:** melatonin, susceptibility to lipid peroxidation, tissue homogenates*

Melatonin (N-acetyl-5-methoxytryptamine) is the main hormone biosynthesized by the pineal gland. This biomolecule coordinates and synchronizes the expression of the most important physiologic sleep-awake effects and biological rhythms. Numerous studies in the scientific literature also describe its multi-regulatory roles in endocrine, immune and nervous systems.[1,3,5,7,11]

Just over a decade ago melatonin was reported to be a free radical scavenger, an antioxidant.[4,13] Lately, the data documenting its ability to overcome oxidative stress has accumulated rapidly. In this regard, the efficacy of melatonin in functioning in this capacity was related to many antioxidant mechanisms: its direct free radical scavenging actions, its ability to enhance the activities of a variety of antioxidative enzymes (catalase, superoxiddismutase)[9], its stimulatory actions on the synthesis of glutathione, its efficacy in reducing electron leakage from the mitochondrial electron transport chain, and its synergistic interactions with other endogenous antioxidants.[18,20,21,22,23]

Moreover, in recent years the experimental studies proved that when melatonin scavenges radicals and related reactants, the products that are generated are also free radical scavengers, thereby greatly enhancing the antioxidant potential of melatonin.[2,6,8,17] Melatonin seems not to undergo redox cycling, it becomes irreversibly oxidized and is considered a suicidal or terminal antioxidant.[1,7]

In this regard, the aim of our study was to evaluate the protective effect (the antioxidant capacity) of melatonin over lipid peroxidation induced in different types of tissues.[8,14,15,16,19,24,25,26]

MATERIALS AND METHODS

Animals

Studies were conducted on 20 male, Albino Swiss mice, weighing 20-22 g. The animals were brought from the 'Carol Davila' University of Medicine and Pharmacy Biobase, and located in the Biochemistry Laboratory of the Faculty of Pharmacy Bucharest. They were housed in a room and maintained at 25 ± 2 °C and 45-55% relative humidity, with an alternating 12h light-dark cycle. They had free access to food and water until the morning of the experiment. All animals used in this study were maintained in facilities fully accredited and the experiments described here were performed in compliance with European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from 2nd February 2002.

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Animals were randomly divided into two working groups, as follows:

-the control group (CONTROL) - 10 mice that received water ad libitum;

-the melatonin group (MEL) - 10 mice for which the drinking water was substituted by a 2 mg/L melatonin solution ad libitum.

The experiment was conducted for 3 weeks, keeping the animals under observation.

Substances

In this study, there were used melatonin and thiobarbituric acid of high purity purchased from SIGMA, USA. Other routine reagents were of the highest purity commercially available.

Sample preparation (tissue homogenates)

After 3 weeks from the beginning of the experiment, animals were sacrificed by decapitation, rapidly excising the targeted tissues: brain and liver. The organs were washed with a 0.15 M NaCl solution, and homogenized 10% (v/v) in a 0.1 M Tris-HCl buffer solution (pH-7.4). The homogenates were centrifuged at 1000 g for 10 minutes and all further determinations were performed on the supernatant.

The susceptibility to autooxidation of the different types of tissue homogenates

The susceptibility of the two types of tissue homogenates to autoxidation was evaluated using the TBARS assay. The assay is based on the reaction of MDA (malonyl dialdehyde) with thiobarbituric acid (TBA), forming a MDA-TBA2 adduct that strongly absorbs at 535 nm. MDA can be found in most biological samples as a result of lipid peroxidation, and has become one of the most widely reported analytes for the purpose of estimating oxidative stress effects on lipids.

The reaction mixture contained: 1 ml tissue homogenate supernatant obtained as previously described and 2 ml 0.1 M Tris – 0.15 M KCl, 1:2 v/v, pH=7.4 solution. The samples were incubated at 37°C, under continuous mixing and the reaction was stopped with TCA (triclor acetic acid) 28%. The amount of lipid peroxides at zero time-T_0 (without incubation) and after 24 hours of incubation-T_{24} was assayed by thiobarbituric acid (TBA) reaction and results were expressed as nmol MDA (malonyl dialdehyde)/100 mg protein. We used an absorbtion molar coefficient of 1.56 $\times 10^2$ mM^{-1} cm^{-1} for MDA at 535 nm. Proteins were measured by Lowry's method.

Statistical analysis

Results were expressed as mean values ± standard deviation. Comparison between groups was carried by use of Student's t. Results were considered statistical significant at $p<0.05$.

RESULTS AND DISCUSSION

Our experiments focused on melatonin's protective effects as antioxidant, as newly quoted in literature.[18,19,22,23]

We investigated the dinamics of cerebral and hepatic tissue susceptibility to autooxidation in two groups of mice: the control group (CONTROL) and the melatonin treated group (MEL). Results were expressed as nmol MDA/mg tissue protein.

Figure 1 shows our results regarding the dynamics of MDA in brain tissue samples from the 2 groups of mice studied: the control group and the one treated with melatonin. MDA levels were monitored initially (T_0) and after 24 hours of incubation at 37°C (T_{24}).

As expected, the MDA levels significantly increase with time, for both groups of mice studied, as follows:

-control group: 0.89±0.2 nmol MDA/100 mg protein (T_0) vs. 1.63±0.31 nmol MDA/100 mg protein (T_{24}); $p<0.02$;

-melatonin group: 0.54±0.14 nmol MDA/100 mg protein (T_0) vs. 1.09±0.28 nmol MDA/100 mg protein (T_{24}); $p<0.01$;

As it can also be observed in figure 1, at zero time (T_0), the melatonin group of mice developed lower amounts of MDA compared with the control group: 0.54±0.14 nmol MDA/100 mg protein vs. 0.89±0.2 nmol MDA/100 mg protein. The same pattern was noticed after 24 hours incubation of brain tissue samples: 1.09±0.28 nmol MDA/100 mg protein vs. 1.63±0.31 nmol MDA/100 mg protein ($p<0.05$). Consequently, at cerebral level, our data showed a

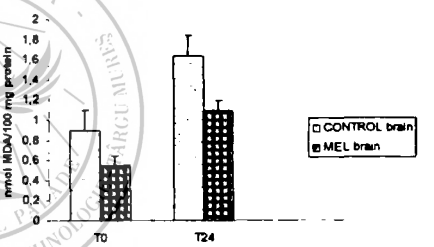

Figure 1. The dynamics of MDA in brain tissue samples at zero time and after 24 hours incubation. The control group of mice and the melatonin group of mice

decrease of lipid peroxidation susceptibility in the group of animals treated with melatonin, both at zero time and after 24 hours incubation.

Figure 2 presents our results regarding the dynamics of MDA in hepatic tissue samples from the 2 groups of mice studied: the control group and the one treated with melatonin. As previously described, MDA levels were monitored initially (T_0) and after 24 hours of incubation at 37°C (T_{24}).

In the case of hepatic tissue, the dynamics of MDA levels showed the same pattern as in brain. In this regard, the MDA hepatic levels significantly increase with time, for both groups of mice studied, as follows:

-control group: 0.34±0.19 nmol MDA/100 mg protein (T_0) vs. 0.6±0.2 nmol MDA/100 mg protein (T_{24}); $p<0.05$;

-melatonin group: 0.29±0.11 nmol MDA/100 mg protein (T_0) vs. 0.5±0.14 nmol MDA/100 mg protein (T_{24}); $p<0.05$;

As it can also be observed in figure 2, at zero time (T_0), at hepatic level, the melatonin group of mice

developed lower amounts of MDA compared with the control group: 0.29±0.11 nmol MDA/100 mg protein vs. 0.34±0.19 nmol MDA/100 mg protein. The same dinamics was noticed after 24 hours incubation of hepatic tissue samples: 0.5±0.14 nmol MDA/100 mg protein vs. 0.6±0.2 nmol MDA/100 mg protein. In another words, in liver, our data showed a decrease of lipid peroxidation susceptibility in the group of animals

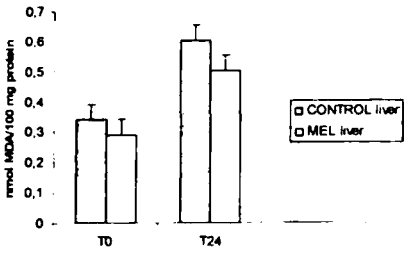

Figure 2. The dynamics of MDA in hepatic tissue samples at zero time and after 24 hours incubation. The control group of mice and the melatonin group of mice

treated with melatonin, both at zero time and after 24 hours incubation.

Figure 3 illustrates, comparatively, the MDA levels, at zero time and after 24 hours of incubation, in brain and liver, for the control and melatonin groups of mice. As it can be noticed, our experimental data show higher amounts of MDA in cerebral tissue, compared with the liver: 0.89±0.2 nmol MDA/100 mg protein vs. 0.34±0.19 nmol MDA/100 mg protein ($p<0.001$).

These results reflect that the physiological level of lipid peroxidation is higher in brain, compared with the hepatic tissue.

Figure 3 also presents, comparatively, the MDA levels at T_{24}, in brain and liver, for the control group of mice. In this case our experimental results show the same pattern as in the case of theinitial state (at zero time): higher amounts of MDA in cerebral tissue, compared with the liver (1.63±0.31 nmol MDA/100 mg protein vs 0.6±0.2 nmol MDA/100 mg proteins; $p<0.05$).

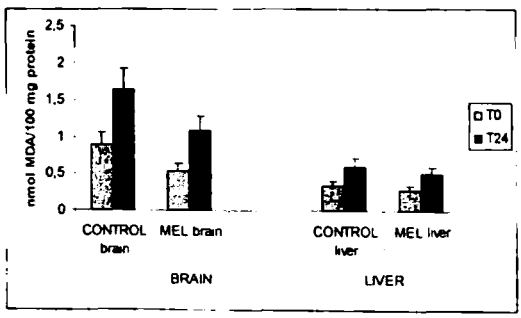

Figure 3. The dynamics of MDA levels, at zero time and after 24 hours of incubation, in cerebral and hepatic tissues. The control and melatonin groups of mice

The data presented in Figure 3 reflect that the physiological level of lipid peroxidation is higher in brain, compared with the hepatic tissue.

Figure 3 depicts, comparatively, the MDA levels at T_0 and T_{24}, in brain and liver, for the melatonin group of mice. As it can be noticed, our experimental results show higher amounts of MDA in brain, compared with the hepatic tissue, both at zero time and after 24 hours of incubation:

-T_0 (melatonin group of mice): 0.54±0.14 nmol MDA/100 mg protein (in brain) vs. 0.29±0.11 nmol MDA/100 mg protein; $p<0.05$;

-T_{24} (melatonin group of mice): 1.09±0.28 nmol MDA/100 mg protein (in brain) vs. 0.5±0.14 nmol MDA/100 mg protein; $p<0.02$.

CONCLUSIONS

Our study on tissue (cerebral and hepatic) lipid peroxidation allowed us to evaluate the prooxidant and antioxidant systems active on tissues (at a certain point) revealing the tissular antioxidant dinamics. In this regard, this parameter is an useful tool for studing tissues antioxidant potential. Its dinamics depends on the oxidation process intensity at cellular level and on the mechanisms involved in the balance between the pro- and anti-oxidant factors.

In this regard, the newest approach of the experimental studies described in literature quotes melatonin (the pineal hormone) as a powerful scavenging biomolecule.

Therefore our experiments focused on cerebral and hepatic MDA levels for studying the influence of melatonin on the dynamics of autooxidation process, namely the susceptibility to lipid peroxidation.

Our results firstly evoked that the cerebral tissue is more vulnerable to lipid peroxidation, compared to the hepatic tissue. This aspect may be easily understood, especially due to the high lipidic content of neuronal cell which makes cerebral tissue prone to oxidative processes.

Our data also succeeded to demonstrate the protective effect of melatonin as antioxidant. The efficacy of this biomolecule proved to be at the highest level in the case of both cerebral and hepatic level.

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Isolation of rat heart mitochondria for in vitro assessment of mitochondrial respiration. Standardization of the experimental model

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The aims of the present study were to develop and standardize the technique of rat heart mitochondria isolation in order to perform in vitro studies of polarographic oxymetry. Oxygen consumption was measured using a Clark type respirometer equipped with an oxygen electrode. The parameters used to measure the complex I dependent respiration (in the presence of substrates glutamate/malate) were State 2 and State 3 respiration rates and the respiratory index calculated as the ratio of State 3 and State 2 oxygen uptake rates. Moreover, cell membrane integrity was checked and mitochondrial function could be further assessed after addition of specific respiration inhibitors or stimulators. The standardized oxymetric technique provides a reproducible method for the assessment of mitochondrial respiratory function. Impairment of respiration in mitochondria isolated at different moments during the postischemic reperfusion will be further addressed in the settings of experimental myocardial infarction in order to develop mitochondria targeted cardioprotective strategies.

Key words: *mitochondria, heart, isolation, respirometry*

Myocardial ischemia/reperfusion (I/R) injury represents a major public health burden, mainly associated with acute coronary syndromes and cardiac surgery. Timely coronary reperfusion by either thrombolysis or primary coronary artery angioplasty has nowadays become the established routine therapy which effectively decreases infarct size and reduces mortality. However, reperfusion is considered a double-edged sword as it can itself induce severe myocardial lesions which paradoxically alleviate the beneficial effects of revascularization.[7] Accordingly, adjunct mechanical and/or pharmacological strategies aimed at limiting cardiomyocyte death by targeting mitochondria beyond reperfusion therapies still represent a priority for both basic and clinical cardiovascular research.[2,5]

Nowadays, it is recognized that mitochondria play a critical role in ischemia/reperfusion related lethal myocardial lesions via the opening of complex pore in the mitochondrial membrane of uncertain molecular identity, known as the permeability transition pore (PTP). During ischemia, PTP is closed, but significant opening occurs at reperfusion in conditions of matrix calcium overload, especially when accompanied by oxidative stress, elevated phosphate concentration and depleted adenine nucleotides.[3] Opening of the mitochondrial PTP pores cause uncoupling of oxidative phosphorylation, massive swelling of mitochondria, rupturing of the outer membrane and cell death through necrosis and/or apoptosis.[4]

Recent experimental data suggest that electron transport modulation in the mitochondrial respiratory chain during early ischemia and/or reperfusion by pharmacological blockage or partial uncoupling of mitochondrial respiration reduce myocardial postischemic reperfusion lesions.[1]

The aim of the study was to standardize the methodology for rat heart mitochondria isolation by the differential centrifugation technique in order to perform respirometry studies in the settings of experimental ischemia/reperfusion injury.

MATERIAL AND METHODS

1. Isolation of Rat Heart Mitochondria

All investigations conformed to the Guide for the Care and Use of Laboratory Animals (US National Institute of Health no. 85-23, revised 1996) and were approved by the Institutional Ethics and Deontology Committee of our university.

Adult Sprague-Dawley rats weighing 250-300 g fed ad libitum and housed at a 12 h light/dark cycle were anesthetized with xylazine (5 mg/kg) and ketamine (20 mg/kg) intraperitoneally. Two animals were used for each experiment and were decapitated 15 min later when unresponsive to noxious stimulation. After thoracotomy, the heart were quickly removed immersed in 4°C cold isolation buffer A (225 mM mannitol, 75 mM sucrose, 1 mM EGTA, 20 mM

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HEPES-TRIS and 0,5 g% bovine serum albumin, pH 7.4). The atria were discarded and the ventricles were minced into 1 mm pieces. The suspension was rinsed and homogenized with a Polytron type tissue homogeniser (ES VELP Scientifica) for 30 sec. in 5 mL isolation buffer A.

Mitochondria were isolated by differential centrifugation (Jouan centrifuge MR23i) at 4°C according to the following protocol: first centrifugation was performed at 1000g for 3 min to remove cellular debris. The supernatant containing the mitochondrial fraction was further centrifuged at 11000 g for 10 min in order to obtain sedimentation of the mitochondrial fraction. The resulting mitochondria pellet was resuspended in 0.5 ml suspension buffer B (buffer A without bovine serum albumin) and centrifuged again at 7000g for 10 min in order to increase the purity of mitochondria. The final pellet was resuspended in 100 µl buffer B and kept on ice.

Total protein concentration was determined by the Biuret method after mitochondria solubilisation with 1% deoxycolate, using bovine serum albumin as standard.

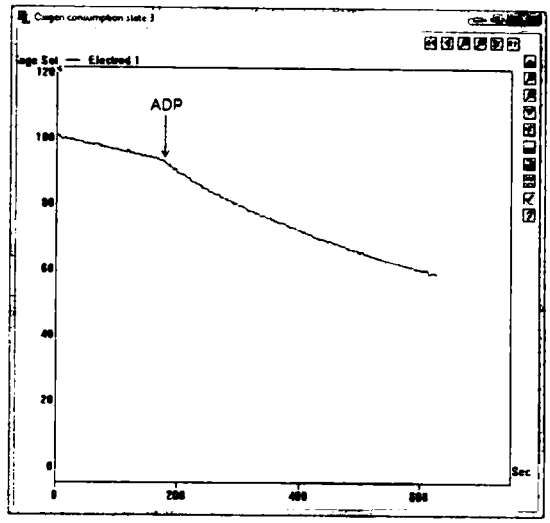

Figure 1. State 2 (in the absence of ADP) and State 3 (after ADP addition, arrow) are depicted

2. Measurement of mitochondrial respiratory rates

Oxygen uptake rates of mitochondria were measured at 25C with a Clark-type electrode (Rank Brothers Ltd., Picolog software) in the incubation buffer C (250 mM sucrose, 1 mM KH2PO4 – Tris, 20 µM EGTA – Tris, 10 mM MOPS – Tris). The solubility of oxygen was taken to be 474 nmolO/mL. Mitochondrial respiration rates were expressed as nanomoles of oxygen per minute per milligram of mitochondrial protein (nmolO/min/mg). The final mitochondrial protein concentration in the Clark electrode chamber for all experiments was 0.5 mg/mL.

Results are expressed as the mean ± S.D.

RESULTS AND DISCUSSIONS

A mean value of 34.26 ± 9.5 mg mitochondrial proteins/mL suspension was obtained when hearts from 2 animals were pooled.

Respiration measurements with Complex I dependent substrates - glutamate/malate (5 mM/2.5 mM, end concentration) were started immediately after preparation of isolated mitochondria. In the beginning we measured the basal respiration rate (V_0 or State 2, in the absence of ADP, i.e. not related to ATP synthesis) for 2 minutes. A mean value (n = 8 experiments) of 56 ± 21 nmolO/min per mg for V_0 was obtained. After that, ADP (250 µM, end concentration) was added and the maximal State 3 respiration (V_{ADP} or ADP stimulated) was recorded for up to 10 min (Figure 1).

The respiration was significantly stimulated by ADP, thus reflecting the capacity of mitochondria to synthesize ATP. Mean V_{ADP} respiration rate was 426 ± 98 nmolO/min per mg (n=8 experiments).

The intactness of the outer mitochondrial membrane was further checked by the subsequent addition of exogenous cytochrome c (25 mM, end concentration) in the metabolic State 4 (V_{CYT}). Cytochrome c is located in the intermembrane space.

The stimulation of respiration by exogenous cytochrome c indicates that endogenous one was lost from mitochondria due to the damage of the outer mitochondrial membrane during the isolation procedure, thus indicating a poor quality of mitochondria suspension. In our hands, the addition of cytochome c did not result in a further increase in respiratory rate, indicating the integrity of the outer membrane (Figure 2).

These results are in agreement with the ones

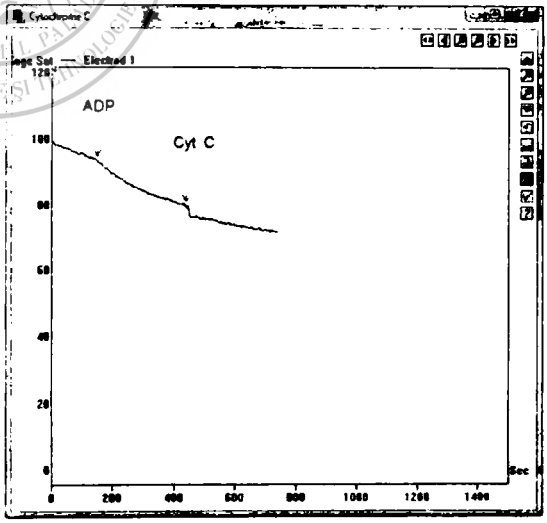

Figure 2. State 3 (ADP stimulated) and the addition of cytochrome c (Cyt) on isolated rat heart mitochondria are indicated with arrows

published by the group of Toleikis who is using the same methos to assess the intactness of the outer mitochondrial membrane. [5,9]

However, the homogenisation phase with the Polytron type tissue homogeniser, if prolonged, can severely affect mitochondria membrane integrity.

The respiratory control index (RCI) or the respiratory control ratio (RCR) was calculated as the ratio of State 3 and State 2 respiration rates. RCR measures the tightness of coupling between electron transfer and oxidative phosphorylation and is the most commonly used parameter to quantify mitochondrial respiration. Our results showed a mean value of 7.6 ± 0.3 for the RCR which is consistent with the data from literature reporting normal values from 6 to 10 with Complex I dependent substrates.[6,8] Ongoing experiments are assessing the differences between respiratory rates in mitochondria isolated from the ischemic area *vs.* the ones isolated form the normal myocardium of the left ventricle in rats subjected to an in vivo model of ischemia/ reperfusion injury.

CONCLUSIONS

Standardization of respirometric measurements on rat heart isolated mitochondria provides a reproducible method for the in vitro assessment of mitochondrial respiratory function. The impairment of respiration in mitochondria isolated at different moments during the postischemic reperfusion will allow us to systematically study the mechanisms involved in mitochondrial dysfunction associated with ischemia/reperfusion injury in order to develop mitochondria targeted cardioprotective strategies in the settings of experimental myocardial infarction.

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Antiepileptic effect of somatostatine measured during low magnesium induced seizure-like activity

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Somatostatine, a widely distributed neuropeptide in the brain and especially in the hippocampus, is associated with seizures both in animal models and human temporal lobe epilepsy. It has been shown that somatostatine containing neurons are sensitive to seizure induced death and it is hypothesized that the loss of somatostatine function in the hippocampus could contribute to epileptogenesis. We recorded extracellular field potentials from neonate rat hippocampus sections perfused with low magnesium artificial cerebrospinal fluid during interictal, preictal and ictal periods of seizures. Than we changed the perfusing solution to one containing 1ëM somatostatine. Somatostatine decreased the length of low magnesium induced seizures by 21.26% (p=0.0298) and increased the interval between them by 11.47% when compared to control. This effect was partially reversible by washout. Because the somatostatine containing neurons are clearly compromised in post-seizure hippocampus and somatostatine appears to have an important antiepileptic effect one can rationalize for the utilization of this drug or its derivates for treating temporal lobe epilepsy. However several caveats are to be considered such as receptor desensitization or disruption of the effect of endogenous somatostatine on the central nervous system.

***Key words**: epilepsy, epileptogenesis, hippocampus, somatostatin*

Epileptic syndromes are one of the most frequent neurological diseases affecting 0.5-3% of the globe's population.[6,8] Almost indifferently of the type of epilepsy the disease will lead to psychopathological complications (hiposexuality, memory loss, depression) as well as the stigmatization of the patients.[1,3] Thus epilepsy causes an important socio-economic burden as well as affecting the quality of life for both patients and relatives. Therefore revealing the mechanisms of epileptogenesis and creating new drug targets is essential and it is worth the intellectual and financial effort.

Somatostatine (SST) is a neuropeptide with an important regulatory role regarding hippocampal excitability. SST has a significant antiepileptic action in rodent models and was associated with human temporal lobe epilepsy.[17] It has been shown that SST containing neurons are sensitive to seizure induced death and it is hypothesized that the loss of SST function in the hippocampus could contribute to epileptogenesis.[12] Experimental observations regarding this effect include: activity-dependent release of SST during seizures, modulation of SST mRNA expression, peptide levels and receptors by seizures and the effect of SST and analogues on seizures.[15]

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EXPERIMENTAL METHODS

Slice preparation. Transverse hippocampal slices (400µm thick), containing the hippocampus proper and the entorhinal cortex, were prepared from the brains of neonate male Wistar rats (10-14 days old). Slices were sectioned at 4°C, transferred to an interface-type storage bath and were incubated in oxygenated artificial cerebrospinal fluid (ACSF, composition in mM: 129 NaCl, 10 glucose, 3 KCl, 1.23 NaH_2PO_4, 1.8 $MgSO_4$, 1.6 $CaCl_2$ and 21 $NaHCO_3$, Ph 7.4) solution for at least one hour at 35°C, and then left to cool down to room temperature (25°C).

Field potential recordings. 400µm thick slices were transferred to a submerged-type recording chamber at 35°C. For field potential (FP) recordings glass microelectrodes (4-10MΩ, pulled from borosilicate glass by a horizontal puller – DMZ Zeitz, Munich, Germany), filled with ACSF were inserted into the CA3 stratum pyramidale. FP signals were amplified (Axoclamp 2B amplifier, Axon Instruments, Foster City, CA, USA) and low-pass filtered at 1kHz with a Cyberamp 320 signal conditioner (8-pole Bessel filter, Axon Instruments, Foster City, CA, USA), and digitized at 20kHz with a Digidata 1320A A/D board (Axon Instruments, Foster City, CA, USA). After the insertion of the electrode the perfusing solution (ACSF) was changed to one containing no added magnesium ions and 5mM potassium in order to induce seizures. This solution is further referred to as low-$[Mg^{2+}]$-ACSF.

After a base recording of seizure-like events somatostatine was added to the solution (1μM) followed by a period of washout with low-[Mg^{2+}]-ACSF. Unless otherwise specified all data will be presented as M±SD (N). A repeated measures analysis of variance (ANOVA) was used to quantify possible temporal trends across discharges and episodes in relation to each other. A value of ≤ 0.05 was considered significant.

RESULTS

We elicited SLEs by low-[Mg^{2+}]-ACSF and after base recordings we added to the solution SST followed closely by a washout period. The characteristics of the recorded SLEs are presented in Figure 1.

The average length of SLEs during base recordings was 80.04±24.34 (14) sec. with an average interval between events of 318.75±106.16 (8) sec. When SST was added to the solution the average length decreased to 63.3±20.36 (14) sec. (by 21.26%, p=0.0298) and the interval increased to 355.33±132.07 (8) sec. (by 11.47%, p=0.2414) – see also Figure 2.

During washout average length increased to 67.27±29.57 (9) sec. (by 6.27%, p=0.3652) and the

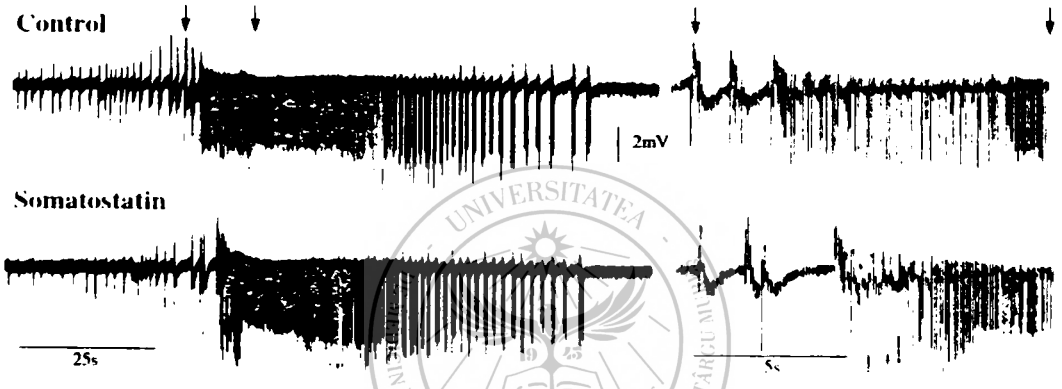

Figure 1. Seizure-like event characteristics during low-[Mg^{2+}]-ACSF and SST containing ACSF perfusion. Note the large number of preictal spikes before the onset of the tonic phase of the seizures and the slowing down at the end of the clonic phase of seizures

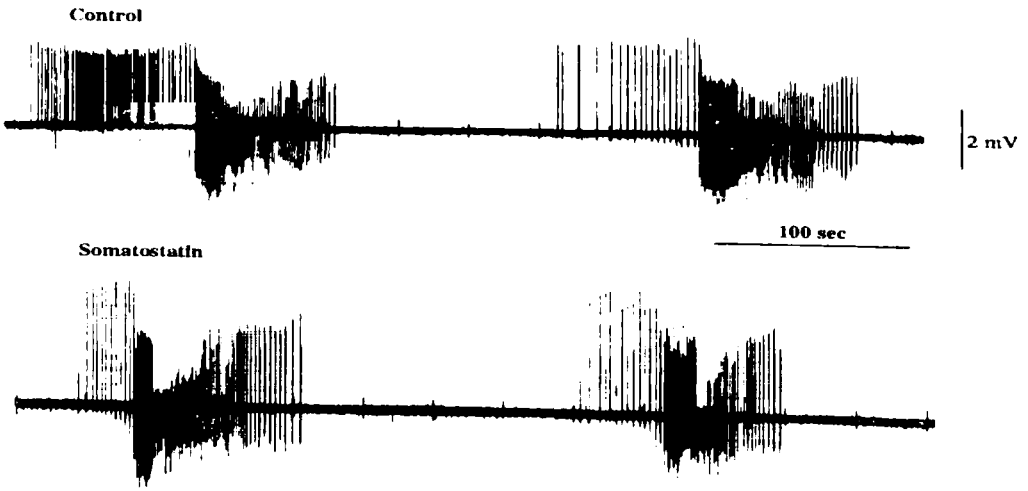

Figure 2. Decrease of length of seizure-like events during perfusion with somatostatine containing low-[Mg^{2+}]-ACSF versus control

free interval between SLEs slightly decreased to 332.01±114.53 (6) sec. (by 6.56%, p=0.3557). Although we did not achieve by washout similar SLE lengths and free intervals as in base recordings (see discussion for details) no statistically significant differences were noted (base compared to washout length decreased by 15.95%, p=0.1489; base compared to washout interval increased by 4.16%, p=0.4104).

DISCUSSION

Our results clearly showed a marked antiepileptic effect of SST on hippocampal SLEs elicited by low-[Mg^{2+}]-ACSF; during SST perfusion seizure length decreased significantly. Although the increase of free intervals between SLEs was not statistically significant a trend could be observed that prompts us to analyze a higher number of sections for statistically more reliable data. The effect of SST on SLEs was only partially reversible; this is probably due to problems related in general to peptide washouts.

It is hypothesized that SST acts specifically on synapses that generate spontaneous epileptiform activity in the CA3 region, the associational/commissural synapses that form a dense network of recurrent interconnections between neurons.[16] Excitatory postsynaptic potentials generated at mossy fiber synapses (the input from dentate granule cells) are insensitive to SST. A major postsynaptic effector for SST receptors is augmentation of I_M (K^+ M current), this is believed to be the likely mechanism contributing to antiepileptic actions of SST.[10] The role of high frequency oscillations (HFO) was not investigated regarding the SST effect although it is known that HFO is present at the onset of the SLE in this model.[9]

There are several reasons to use SST related targets for treating intractable human temporal lobe epilepsy: (1) the somatostatine containing neurons are clearly compromised in post-seizure hippocampus and (2) somatostatine appears to have an important antiepileptic effect. However, many problems are to be considered, these caveats usually apply to any neuropeptide system as well. First, one needs to consider the possible disruption of endogenous SST effects. This is a significant issue as SST plays an important role in cognition[14], food intake[13], fear conditioning[7] and growth hormone regulation.[2] Because SST is widely distributed in the brain targeting all SST receptors would surely disrupt the endogenous effect. Unfortunately, although we know that the major antiepileptic effect comes through $SSTR_2$, this receptor appears to be the most important receptor in all human (and rat) brain regions. Studies in mice revealed a similar antiepileptic effect on [11]$SSTR_4$ but this effect has not been proved in humans nor rats. Another issue is receptor desensitization. Even if SST is endogenously released $SSTR_2$ is sensitive to downregulation.[5] During seizures because of the large amounts of SST released there is a sustained reduction of SST receptors.[4] Despite of this it is hypothesized that it might be possible to design an appropriately selective synthetic SST agonist with a low propensity for receptor downregulation.[15]

The high number of patients with therapeutically refractory temporal lobe epilepsy and the limited number of peptides/proteins on which the existent antiepileptic drugs act suggests validation of new targets. Therefore it is important to examine the effects of $SSTR_2$ and $SSTR_4$ agonists both at in vitro and in vivo experimental settings and also to validate the actions of SST and agonists in human hippocampus.

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Colon adenoma-carcinoma sequence: E-cadherin/ syndecan-1, Ets-1/matrix-metalloprotease-7

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Introduction: E-cadherin, syndecan-1, Ets-1 and MMP7 are candidate markers of colorectal cancer, playing a role in carcinogenesis follow-up. Our goal was to perform a comparative immunohistochemical study of these markers in different stages of the adenoma-carcinoma sequence. Materials and methods: We processed colon adenoma and carcinoma biopsies and resected tumors from the Pathology Laboratory of the Clinical County Hospital of Târgu-Mureş, Romania. We used the Ultravision Labeled Polymer and labeled streptavidin-biotin complex methods. The immunohistochemical results were immunohistochemically graded, and used for determination of different immune phenotypes that have been compared by statistical tests. Results: 74% of adenomas are Ecad+/syn+, and 66.6% are Ets1-/MMP7+. 75% of carcinomas are Ecad-/syn-, and 8.3% are Ets1-/MMP7-. Conclusions: During the evolution of the carcinogenetic process E-cadherin and syndecan-1 immunoexpression displays a progressive decrease, and Ets-1 immunoexpression a progressive increase. MMP7 immunoexpression appears in early stages and persists through the sequence.

***Key words**: immunohistochemistry, colon adenoma, colon carcinoma*

The existence of the adenoma-carcinoma sequence has been confirmed by many studies performed on colorectal carcinomas (CRCs), and lately it became the model for malignant transformation of epithelial tissue. The transformation of adenomas with low-grade and moderate dysplasia into those with high-grade dysplasia ("carcinoma in situ"), and into invasive and metastatic carcinoma is associated with several molecular alterations, i.e. oncogene activation and oncosupressive gene inactivation.[14] Genetic and epigenetic changes in adenomas are similar to those described in carcinomas.[10]

E-cadherin is a 120 kDa glycoprotein, and is one of the calcium dependent intercellular adhesion molecules (CAM).[12] Decrease of E-cadherin expression in human tumors is followed by loss of the normal epithelial morphology and increased metastatic potential of malignant cells.[8]

Syndecan-1 (CD138) is expressed on the basolateral surface of epithelial cells, endothelial cells and vascular smooth muscle cells.[4] Decrease of syndecan-1 expression is followed by changes in cellular morphology, motility, growth and differentiation, and an epithelial-mesenchimal transformation takes place.[5]

Ets-1 protein is a transcription factor expressed in the stroma of invasive processes.[16] It takes part in matrix-metalloprotease (MMP-1, MMP-2, MMP-3, MMP-7, MMP-9) activation, thus playing a role in tumor invasion.[3,16]

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Matrix-metalloproteases (MMPs) are zinc dependent endopeptidases, playing a role in extracellular matrix and basal membrane degradation.[1] MMP7 is a matrilysin expressed by CRC tumor cells, and plays a role in tumor invasion.[2] It has been shown that MMP7 is involved in shedding of syndecan-1 and E-cadherin.[9,15]

The aim of this study was to determine the expression of these markers trough the adenoma-carcinoma sequence, using immunohistochemical methods.

MATERIAL AND METHODS

In this study we included 27 colon adenomas and 36 colon carcinomas, from the Pathology Department of the Clinical County Hospital of Târgu-Mureş. We used the following mouse monoclonal antibodies: Lab Vision anti CD138 Ab-2 (MS-1793), concentration 1/50, Lab Vision anti E-cadherin SPM471 (MS-9470), concentration 1/200, Lab Vision ETS-1 Ab-1 (1G11), concentration 1/20, and Chemicon International anti-MMP7 (MAB3315), concentration 1/500. For E-cadherin, syndecan-1 and Ets-1 the immunostaining method used was Ultravision Labeled Polymer, and for MMP7 it was the labeled streptavidin-biotin complex method.

We determined the frequency of immunopositive cells (FIC) and the signal intensity (I). We established the following scoring system: FIC 0: 0-5%, FIC 1: 6-30%, FIC 2: 31-75%, FIC 3: 76-100%; and the signal intensity: absent, weak, moderate, strong. Based on these results we determined the following phenotypes: Ecad-/syn- or absent or decreased immunoexpression (FIC 0-2), Ecad+/syn+ or unmodified immunoexpression

(FIC 3); Ets1-/MMP7- or absent immunoexpression (FIC 0), Ets1+/MMP7+ or positive immunostaining (FIC 1-3).

The immunohistochemical results were correlated using the GraphPad In Stat 3 statistics software (version 3.06), by Pearson, chi-square, Fisher and student tests. The obtained values were considered statistically significant in case of $p<0.05$ and a 95% confidence interval.

RESULTS

In normal tissue E-cadherin and syndecan-1 expression is found in the cell membrane, and in tumor tissue in the cell membrane and cytoplasm. In tumor cells Ets-1 has a nuclear expression, and MMP7 is found in the cell membrane and cytoplasm. These are absent in normal epithelia.

In adenomas E-cadherin and syndecan-1 had unmodified immunoexpression in 85.1% (23), and 81.4% (22) respectively. In carcinomas these values were 25% (9), and 8.3% (3) respectively. In adenomas Ets-1 and MMP7 immunoexpression was present in 18.5% (5), and 81.5% (22) respectively. In carcinomas these values were 58.3% (21), and 80.5% (29) respectively.

The majority of adenomas (74%) were Ecad+/syn1+ (Table I), and 75% of carcinomas were Ecad-/syn1- (Table II) ($p< 0.0001$). 66.6% of adenomas were Ets1-/MMP7+ (Table I), and 47.2% of carcinomas were Ets1+/MMP7+ and 33.3% Ets1-/MMP7+ (Table II) ($p=0.01$).

The Ecad-/syn1- immunophenotype was more frequent in tubulovillous adenomas ($p=0.4$), with high-grade dysplasia ($p=0.02$), and located in the left colon ($p=0.1$). In case of carcinomas this immunophenotype was found especially in poorly differentiated or undifferentiated adenocarcinomas ($p=0.2$), mucinous adenocarcinomas ($p=0.9$), that were stages $pT_{3,4}$ ($p=0.6$), $pN_{1,2}$ ($p=0.9$) and Dukes's stage C-D ($p=0.07$). These were located in the right colon ($p=0.8$), were bigger than 40 mm ($p=0.6$), and developed in males ($p=0.05$) of older age (over 60 years, $p=0.6$).

The Ets1+/MMP7+ immunophenotype was found especially in tubulovillous adenomas ($p=0.3$), with moderate dysplasia ($p=0.1$), located in the left colon ($p=0.5$), and developed in males ($p=0.1$). In case of carcinomas this immunophenotype was found especially in poorly differentiated or undifferentiated carcinomas ($p=0.8$), without mucinous component ($p=0.5$), that were stages $pT_{2,4}$ ($p=0.9$), $pN_{1,2}$ ($p=0.7$) and Dukes's stage C-D ($p=0.6$). These were located in the right colon ($p=0.8$), were bigger than 40 mm ($p=0.3$), and developed in males ($p=0.3$) over 60 years of age ($p=0.8$).

We noticed a progressive and statistically significant increase of cases with decreased or absent E-cadherin and syndecan-1 ($p<0.0001$) through the adenoma-carcinoma sequence (adenoma without dysplasia, adenoma with low-grade, moderate and high-grade dysplasia; well, moderately and poorly differentiated and undifferentiated adenocarcinoma) (Figure 1). This could not be demonstrated in case of Ets-1 and MMP7 positive cases ($p=0.3$) (Figure 2).

There is a positive correlation between E-cadherin and syndecan-1 expression of adenomas and carcinomas. Ets-1 and MMP7 immunoexpression displays a negative correlation in adenomas, and a positive one in carcinomas. (Table III)

Table I. Number (percent) of different immunophenotype cases in adenomas

Adenomas	Ets1+ / MMP7+		Ets1-/ MMP7+		Ets1+ / MMP7-		Ets1- / MMP7-		Total	
	n	%	n	%	n	%	n	%	n	%
Ecad+/syn1+	3	*11.1*	14	*51.8*	0		3	*11.1*	20	*74*
Ecad+/syn1-	0		1	*3.7*	1	*3.7*	1	*3.7*	3	*11.1*
Ecad-/syn1+	0		2	*7.4*	0		0		2	*7.4*
Ecad-/syn1-	1	*3.7*	1	*3.7*	0		0		2	*7.4*
Total	4	*14.8*	18	*66.6*	1	*3.7*	4	*14.8*	27	*100*

Table II. Number (percent) of different immunophenotype cases in carcinomas

Carcinomas	Ets1+ / MMP7+		Ets1-/ MMP7+		Ets1+ / MMP7-		Ets1- / MMP7-		Total	
	n	%	n	%	n	%	n	%	n	%
Ecad+/syn1+	2	*5.5*	0		0		1	*2.7*	3	*8.3*
Ecad+/syn1-	1	*2.7*	3	*8.3*	1	*2.7*	1	*2.7*	6	*16.6*
Ecad-/syn1-	14	*38.8*	9	*25*	3	*8.3*	1	*2.7*	27	*75*
Total	17	*47.2*	12	*33.3*	4	*11.1*	3	*8.3*	36	*100*

Table III. Correlations between E-cadherin and syndecan-1, and Ets-1 and MMP7 immunohistochemical results in carcinomas and adenomas (CC - Correlation coefficient; CI - Confidence interval)

	Ecad/syn1		Ets1/MMP7	
	CC	CI	CC	CI
Adenomas	0.23	-0.16-0.56	-0.05	-0.42-0.33
Carcinomas	0.73	0.53-0.85	0.12	-0.21-0.43

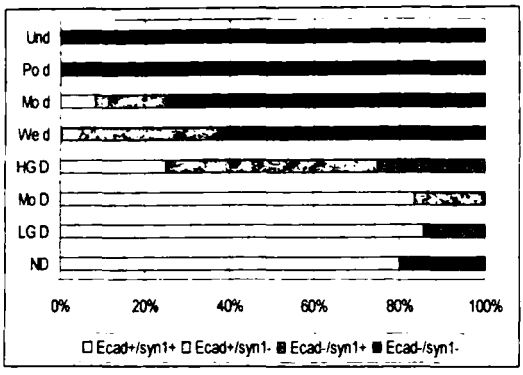

Figure 1. Correlation of E-cadherin/syndecan-1 immunophenotypes with adenoma grade of dysplasia and carcinoma histological grade (ND- non-dysplastic adenomas; LG D- adenomas with low-grade dysplasia; Mo D- adenomas with moderate dysplasia; HG D- adenomas with high- grade dysplasia; We d- well differentiated adenocarcinoma; Mo d- moderately differentiated adenocarcinoma; Po d- poorly differentiated adenocarcinoma; Und- undifferentiated carcinoma)

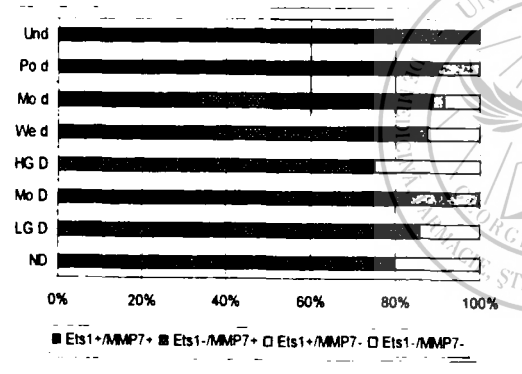

Figure 2. Correlation of Ets-1 /MMP7 immunophenotypes with adenoma grade of dysplasia and carcinoma histological grade (ND- non-dysplastic adenomas; LG D- adenomas with low-grade dysplasia; Mo D- adenomas with moderate dysplasia HG D- adenomas with high-grade dysplasia; We d- well differentiated adenocarcinoma; Mo d- moderately differentiated adenocarcinoma; Po d- poorly differentiated adenocarcinoma; Und- undifferentiated carcinoma)

DISCUSSION

Our study is the first to try to establish a new immunophenotype by evaluating the E-cadherin/ syndecan-1 and Ets-1/MMP7 expression in colon adenomas and carcinomas.

E-cadherin and syndecan-1 expression is present in majority of adenomas and decreases significantly in adenomas with high-grade dysplasia and in carcinomas. Thus, it displays a progressive and statistically significant decrease through the adenoma-carcinoma sequence. MMP7 expression is present in both adenomas and carcinomas, and Ets-1 immunoexpression occurs more frequently in carcinomas. Thus, MMP7 expression occurs in early stages, while Ets-1 expression in late stages of carcinogenesis.

Only few immunohistochemical studies exist that compare these markers in colon adenomas and carcinomas. In 1999, Richard et al.[13] compared syndecan-1 and E-cadherin immunoexpression in colorectal adenomas and carcinomas. Their results are similar to ours: E-cadherin expression decrease in carcinomas compared to adenomas correlated with decreased syndecan-1 expression, while the latter was less intense. This decrease was more pronounced in adenomas with moderate dysplasia compared to adenomas with high-grade dysplasia, and in adenomas compared to carcinomas. Based on these results the authors proposed that syndecan-1 expression changes precede similar changes of E-cadherin.

In 2006, Fernanda et al.[7] demonstrated an E-cadherine and membrane beta-catenin expression decrease in CRCs compared to normal tissue. Along with the membrane beta-catenin decrease, an increase in its nuclear expression has been observed. Out of MMP2, 7, and 9 the MMP7 expression had the highest intensity and frequency in CRC. Its expression correlated with nuclear beta-catenin expression and was associated with reduced survival rate of the patients. Another study performed in pT3 CRCs demonstrated a positive correlation between cytoplasmic and nuclear expression of beta-catenin and cytoplasmic expression of MMP7, and membrane and cytoplasmic expression of E-cadherin and beta-catenin.[6]

In 2008, Ochiai et al.[6] established a new formula based on the immunohistochemical grading of dysaderin, E-cadherin and MMP7. This method can be used for detecting hepatic metastases with 87% sensibility and 66.5% specificity.

We concluded that E-cadherin and syndecan-1 immunoexpression decreases, and Ets-1 immunoexpression increases through the adenoma-carcinoma sequence. MMP7 expression is present in early stages of carcinogenesis and takes part in cleavage of E-cadherin and syndecan-1 dependent intercellular adhesions.

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Implication of cardiovascular risk factors in coronary disease in women

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Aim: Because in women the incidence coronary disease rises significantly after the installation of menopause, around the age of 65 of years, we tried to observe the implication of the cardiovascular risk factors in the coronary pathology in premenopausal and menopausal women .

Material and method. We considered in-patients in Clinica Medicala III suspiciously of the complaint coronary, in the period January 2000 December 2007, classified in two groups: Group A of 190 of patients in premenopause with an average age of 50±3, 3 years, group B 210 patients in the first years of menopause, with an average age of 58±4, 5 years, where we evaluated the cardiovascular risk factors: Hypertension, personal medical history, family history, obesity, diabetes, dyslipidemia, smoking as well as their association.

Results. We obtained the following data: The coronary disease was present in 45% of the perimenopausal patients and in 60% in patients at menopause(p= 0, 0012), in perimenopauseal patients with CHD 92%presented high blood pressure, 61% obesity, 78% dyslipidemia, 13% hyperglycemia, 13% smoking, 14% positive medical history, in most cases with the association of 2 risk factors; In menopause, the risk factors revealed were High blood pressure 94%, obesity 79%, dyslipidemia 90%, hyperglycemia 26%, smoking 15%, history of cardiovascular diseases 22%, the majority with 3 risk factors.

We haven't found any statistically significant differences between the two groups regarding hypertension, the personal and family medical history and smoking. In the menopause group there is a significant increase in the number of patients with obesity, dyslipidemia and hyperglycemia, as well as the association of more cardiovascular risk factors.

Conclusions: In women at menopause there is an increase in the incidence of obesity, dyslipidemia, hyperglycemia, as well as the association of more cardiovascular risk factors causing a statistically significant increase in the incidence of coronary disease.

***Key words**: cardiovascular risk factors, premenopausal and menopausal women*

The epidemiologic studies realized in the past decades have shown that coronary disease represents the main cause of death in woman above the age of 65 years, both in developed countries and in developing countries, exceeding the mortality through breast or uterine cancer and thus becoming a problem of public health.[1,13] Unfortunately not only the women above 65 years present severe forms of cardiovascular disease. Minimizing the importance of coronary disease in women has its origin partly in the results of the Framingham study, whose data was acquired several decades ago, and thus reflects an old situation, and not the present prevalence of coronary disease in women. Around the age of 50 years, the rapid rise of cardiovascular disease incidence in women seems to be better correlated with the menopause inducted estrogen level decrease, rather than the age. Taking in account this epidemiological data, as well as clinical findings in respect to cardiovascular disease in women, and the earlier age of debut in the later period, this studies objective was the evaluation of: the incidence of coronary pathology in women in perimenopause, and in the first years after menopause onset, until 65 years of age; as well as the incidence of cardiovascular risk factors, the importance of their accumulation, and the impact on various forms of coronary disease, this life stage accumulating the effects of cardiovascular risk factors and the decrease in estrogen level which starts in the premenopausal period.

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MATERIAL AND METHODS

Between January 2000 and December 2007, we realized a study with selected patients, admitted to Clinica Medicala III Târgu-mures with the suspicion of coronary disease and which presented thoracic pain, multiple cardiovascular risks factors, resting electrocardiographic modifications and during stress tests, placed in two groups:

-220 patients in perimenopause,

-240 patients in the first years of menopause.

Patients evaluated in this study went through a diagnosis protocol containing: anamnesis, physical examination, laboratory tests and complementary examinations.

From data obtained through anamnesis we retained for analysis and interpretation the following results:

-medical family history cardiovascular diseases present in relatives of 1st degree under 55 of years in men and under 65 of years in women

-smoking recording if the patient was or still is a smoker

-hypertension if blood pressure values were equal or higher than 140/90mmHg, 130/80 in patients with diabetes mellitus and/or the patient was under blood pressure lowering medication with the hypertension grade previously specified

-dyslipidemia was diagnosed if the patient had elevated lipid levels or was under lipid lowering therapy.

-Diabetes mellitus was certified if the patient with elevated fasting glucose or if under specific treatment

-Obesity – patient were considered obese if the body mass index was over 30kg/m^2, with android type obesity

RESULTS

In the present study we found that ischemic hearth disease appears more frequently in menopause, a decade later than in men, but its incidence starts to increase already from the premenopause period, starting with the decline of estrogen hormones; its incidence in premenopause 45%, and in menopause 60%, a statistically significant difference (p= 0,0012).

Family history. In our study, anamnestic data suggest the presence of family history of cardiovascular disease in 24% of patients with coronary disease in premenopause and in 23% in menopause. However we found a statistically significant difference (p=0.049), regarding family history of cardiovascular disease in younger patients, with coronary disease in perimenopause24% versus 14% in patient without coronary disease.

Smoking In respect of the prevalence of this cardiovascular risk factor we found that 14% in patients in premenopause were smokers and 12% in patients at menopause. At menopause 15% of patients with coronary disease were smokers, while in the group without coronary disease smoking was present in 7.5% (p=0.07)

Hypertension. In our study the risk factor with the highest incidence both in patients in perimenopause 94% and in those at menopause 95%.

Obesity. Was frequently encountered In our study with a statistically significant difference (p=0,000002), between the incidence of obesity in patients in premenopause 57,5% and patients at menopause 78%. Regarding obesity in patients with coronary disease we found a statistically significant difference (p=0,0027), patients in premenopause having the incidence of obesity of 61,5% and patients at menopause 795 incidence of obesity.

Diabetes mellitus. The incidence of diabetes mellitus in our study was: 14% of the patients in premenopause had diabetes mellitus, 2.7% of which needed insulin therapy and 11.3% were on oral medication. In patients at menopause the incidence of diabetes mellitus was 23%, 4% on insulin therapy and 19% on oral medication, this difference being statistically significant (p=0.032).

A statistically significant difference (p=0.0329) was found regarding the incidence of diabetes mellitus in patients in premenopause without coronary disease 19% compared to patient with coronary disease which had an incidence of diabetes mellitus of 26%.

Dyslipidemia is the second most frequent cardiovascular risk factor encountered in our study with very high ratios. Dyslipidemia is present in 65.5% in patients in premenopause, and in 83% in patients at menopause with statistical significance (p=0.000016). Dyslipidemia has also a significantly higher incidence (p=0.0002) in patients in premenopause with coronary heart disease up to 79% compared with patients without cardiovascular disease that had dyslipidemia in only 55%. The same statistical significance (p=0.0002) was found in patients at menopause with very high incidence of dyslipidemia in patients with coronary disease compared with 72% in patients without coronary disease.

Risk factors association. Regarding the association of cardiovascular risk factors we found a statistically significant difference (p=0.005) with a high percentage of patients with 3 or 4 risk factors association in patients at menopause compared with those in premenopause who had 2-3 risk factors association. In our study patients with coronary disease and 3 cardiovascular risk factors association were present in 60% in premenopause and 76% in menopause.

DISCUSSIONS

If conventionally ischemic heart disease was considered to affect mainly males the female population being poorly represented in cardiovascular research, epidemiologic studies performed in the last decade have shown that female patients represent a well defined subpopulation among patients with coronary disease, coronary heart disease representing the main cause of death in women over 65 years both in developed and developing countries. Although in both male and females the cardiovascular risk factors are the same, their impact on individual risk is different in women where specific hormone levels seem to modulate their effect.

A family history of cardiovascular disease is considered as an independent risk factor in women for coronary disease. Unlike the previous studies[4,5,21] that minimized the importance of this risk factor in women with coronary disease, recent studies[19,25] have shown that positive family history represents a major risk factor for these patients, and women with significant family history have a high risk of premature coronary events. The Nurse's Health Study which contained over 120000 women under 55 years of age, found that females whose parents suffered a myocardial infarction before the age of 60 years presented a relative risk of 5, compared with women whose family history contain myocardial infarction over 60 years who had a relative risk of 2.6.[3] Our study has also shown significant family

history present in premenopausal women with coronary disease compared with patients without cardiovascular disease.

Smoking is along dyslipidemia, and hypertension a major changeable risk factor that doubles the mortality rate. The adverse effect of smoking is directly proportional with the smoking duration.[25,33] Although due to the educational measures the number of smokers seems to decrease, the number of female smokers tends to rise and fewer women quit smoking.[9] The association of smoking and the different forms of coronary disease and in particular acute myocardial infarction has been documented by numerous studies.[2,9]

The lower incidence of smoking as a risk factor, found in our study is possibly due to certain psychosocial reserves in admitting this habit due to the pejorative perception of smoking in women, thus some patients didn't admit they had this habit. In a prospective study that included both males and females below 55 years, has been shown that the incidence of myocardial infarction increases 6 times in smoking women, and only 3 times in smoking men. In Nurse's Health Study by comparing smoking and non smoking women it was found that the relative risk increases 3 times in women smoking 1-4 cigarettes/day. This risk increases to 4 times if the number is between 15-24/ day and to 6 times if more than 25 cigarettes/day are smoked.[26]

Hypertension. Although before the age of 45 the prevalence is much larger in men, after this age hypertension and especially systolic hypertension presents a significant increase especially in women, so much so that at the age of 60 it affects them in a proportion of 70%.[27] This fact lead to the introduction for the first time of a separate chapter "hypertension in women" in the 2007 Hypertension Management Guidelines elaborated by the European Society Of Hypertension and the European Society Of Cardiology.

The high incidence observed in our study, over 90% hypertension at a relatively young age, our patient hawing under 65 years of age, is due to the fact that they are a selected population, that were referred to the cardiology specialist with the suspicion of coronary disease and multiple risk factors.

A Swedish study run for 12 years on 1462 women has shown that the incidence of AMI is higher in patients with hypertension even if under treatment, compared to those with normal blood pressure.[1,8], and a US study in which 57% of the total patients were women, has shown that an effective antihypertensive therapy reduces the incidence of coronary disease by 25%.[12] Most published studies[1,8,28] report blood pressure increase in the elderly. Our study shows a high incidence of hypertension in the adult women with coronary disease, underlining the importance of this risk factor in this category of patients.

Obesity is an independent risk factor for coronary disease in both sexes. Nurse's Health Study with almost 120000 women observed for 14 years, shown a positive connection between obesity and coronary disease. Thus women with a 20 Kg weight gain had an increase in coronary risk of 2.7 times larger than women with a weight gain of only 5 Kg. The Women's Ischemia Syndrome Evaluation – WISE study, shown that 27% of women were subjected to weight variations [24, 35]. Although studies have attributed a positive effect to the weight loss in the obese, weight fluctuations are associated with an increase in coronary risk, the WISE study reporting low levels of HDL cholesterol in women with large weight variation, either way. In our study the incidence of obesity was significantly larger in patients with coronary disease at menopause compared to those without coronary disease

Diabetes mellitus is for both sexes a major cardiovascular risk factor[15,20,34], but in women it has a special significance by removing the advantage of the female sex, thus compared with diabetic men that have a 2-3 times increase in cardiovascular risk, diabetic women have a 3-7 times increase in cardiovascular risk.[10,16,22]

Although both men an women tend to develop diabetes at the same age, and diabetes mellitus increases the risk of fatal coronary events regardless of sex[30,31], data from the Framingham study and a meta-analysis of 10 studies[13,14,22] suggests that diabetes mellitus is a risk factor of higher significance in women, with more serious consequences than in men, the relative risk in women being 2.58 while in men it is 1.85, and the fact that in diabetic women the protective effects of estrogens are diminished or even cancelled. Based on this data the Adult Treatement Panel III established DM as an equivalent for coronary disease[11] and recommended aggressive antiatherosclerotic therapy combined with the diabetes treatment itself, and hypertension treatment. In our study the incidence of DM was significantly higher in patient with coronary disease at menopause.

Dyslipidemia. The large majority of studies that demonstrate a correlation between the higher values of total cholesterol, its fractions and the incidence of coronary disease were effectuated in men[6], and the extrapolation to women is very difficult, if we consider that the decrease of estrogen levels after menopause alters the lipid profile, therefore increasing the risk of coronary disease.[10] In our study dyslipidemia, was second most frequent risk factor after hypertension, with statistically significant differences in patients with coronary disease compared to those without hart disease, both in premenopause and in menopause.

In the last years, studies performed on women indicate a low level of correlation between the incidence of coronary disease and the values of total cholesterol and of LDL-cholesterol in women under 65 years. An important role is attributed to HDL-cholesterol whose decrease under 35 mg/dl, can double the risk of myocardial infarction in woman compared to men with similar values. Many clinical studies [6, 10, 17] they have shown the fact that in women the strongest prognosis factor is the level of HDL-cholesterol as well as the ratio of total cholesterol/ HDL-cholesterol. According to the guide elaborated by the National

Cholesterol Education Project from the USA these two parameters are used as screening tests for the evaluation of coronary disease risk in women.

An important finding concerning the coronary disease is that most frequently there is an association of risk factors. Thus, in the Framingham study less than 20% of women had only hypertension; the remainder of hypertensive woman hawing associated other cardiovascular risk factors as well. While in men the coronary events were associated to 2 or more risk factors in 30% of cases, in women this proportion is much higher, about 70% of cases[32], similar to the results from our study.

CONCLUSIONS

In this study we wished to evaluate, both in premenopause and menopause, the incidence of risk factors, the importance of their aggregation, the impact they have on various clinical forms of coronary disease. Although similar with data from literature, the conclusions from our study reveal some particular aspects.

Ischemic heart disease in women appears more frequently in menopause, with a decade later than in men, but its incidence starts to rise in premenopause, coinciding with the decline of the estrogen hormones, as we have also seen in this study, the incidence in premenopause is 45%, and at menopause 60%, a statistically significant difference.

In respect of the cardiovascular risk factors we found

-The high incidence of positive family history, also found in patients from our study, mainly in the younger patients as well as in acute coronary syndromes,

-The low incidence of smoking in our study is determined by a psychosocial reserve in anamnestic recording, some patients not admitting this habit;

-The prevalence of hypertension in women suddenly increases after the age of 45 years, and is the most frequent risk factor in our study

-The incidence of obesity in patients with coronary disease was significantly higher in women at menopause

-Diabetes mellitus had a significantly higher incidence in patients with coronary disease at menopause, especially the acute coronary syndromes

-Dyslipidemia is the second most frequent risk factor with a very high incidence in patients with coronary disease

-In women the association of 2 or more risk factors in perimenopause and 3 or 4 at menopause is encountered in a very high proportion 70%.

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Incidence of single vessel coronary artery disease in patients with new-onset angina pectoris

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Background. New-onset angina pectoris is part of the unstable angina pectoris group, that reunites several clinical forms of angina, having in common a severe myocardial ischaemia. The purpose of the study. The aim of the study was to evaluate the coronary injuries in patients clinically diagnosed with new-onset angina pectoris. Material and methods. 165 patients were enrolled (beeing addmitted between January 1st and December 31st 2007). The patients belonged to all age groups and they were all clinically diagnosed with new-onset angina pectoris. All patients underwent diagnostic coronarography. Patients with valvular diseases, cardiomyopathies and cardiac failure were excluded. We evaluated dynamic electrocardiographic changes, coronarographic location and severity of coronary injuries. Results. Out of 96 patients with more or less significant coronary lesions, 58 had single vessel disease, 21 had bivascular coronary disease and 17 had multiple vessel disease. Electrocardiographic changes were not significantly correlated with the severity of the coronary injuries. The most frequent ECG change was the negative T wave (39 percent of the patients). Discussions, conclusions. Our results show that patients with new-onset angina have an unusually large incidence of single vessel coronary artery disease, predominantly involving the left anterior descending coronary artery. A significant number of patients with new-onset angina present severe coronary stenoses and multiple vessel lesions. The present study did not succeed in finding a statistically significant correlation between ECG changes and the severity of coronary lesions. Thus, coronary angiography remains the criterion standard for diagnosing and evaluating the extent and severity of coronary artery disease.
***Key words:** new-onset angina pectoris, electrocardiogram, coronary stenosis.*

Resting electrocardiogram (ECG) has a limited contribution to the diagnosis of angina pectoris, considering the high frequency of normal tracings. In resting conditions, 30-60% of patients with angiographic confirmed coronary artery disease, have normal ECG. If the pathological phenomenon does not influence the myocardial electrogenic processes, the electrical traces remain inexpressive, so futile.[1,2,6] Coronarography represents the most useful method in evaluating the extent and severity of coronary artery disease, especially when corroborating the data from left ventriculography. Thus, the obtained information is indispensable for assessing the opportunity for myocardial revascularization and prognostic evaluation.[3,4,5]

The aim of the study was to evaluate the coronary lesions in patient with new-onset angina and to establish the correlation between ECG changes and the severity of the coronary injuries.

MATERIAL AND METHOD

We conducted a retrospective study of 165 patients with new-onset angina, admitted to the Adults Cardiology Clinic I of The Emergency Institute of Cardiovascular Diseases and Transplant, Targu Mures, between January 1st and December 31st 2007. We enrolled patients without known cardiac history, accusing symptoms suggesting coronary artery disease (angina), with recent onset (<2-3 months). Only patients with performed coronarography were included, and age limit was not taken into account. Patients with known valvular diseases, cardiomyopathies and those with heart failure, clinically or echocardiographically diagnosed (left ventricle ejection fraction less then 50%), were excluded. Reference data: clinical symptoms, presence of known cardiovascular risk factors, resting ECG traces, echocardiographic findings, coronaroangiography.

CHI test, Student test and Fisher Exact test were used for statistical evaluation.

RESULTS

Out of 96 patients with more or less significant coronary lesions, 58 had single vessel disease, 21 had bivascular coronary disease and 17 had multiple vessel disease. Patients with single vessel disease did not differ from patients with multivessel involvement with respect to risk factors or clinical presentation. A significant number of patients presented severe coronary stenoses and multiple vessel lesions. (Figure 1, 2)

An important number of patients (67%) presented major ECG changes: inverted T waves; depressed ST segment; depressed ST segment with inverted T waves; conduction disturbances; elevated ST segment. (Figure 3).

The most frequent change encountered in resting ECG traces in patients with new-onset angina (Table I).

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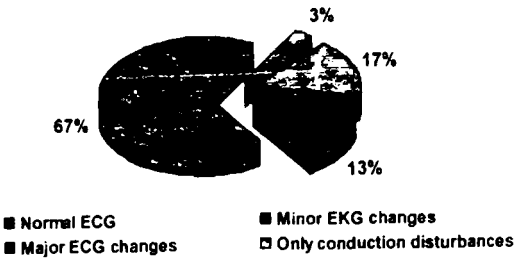

Figure 1.

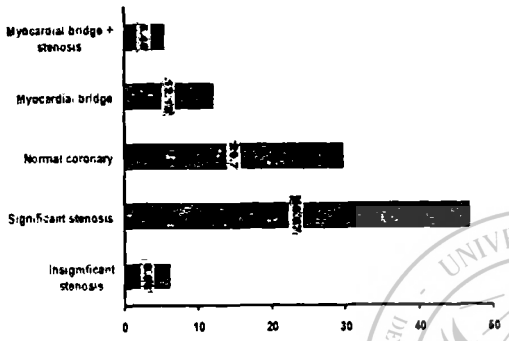

Figure 2.

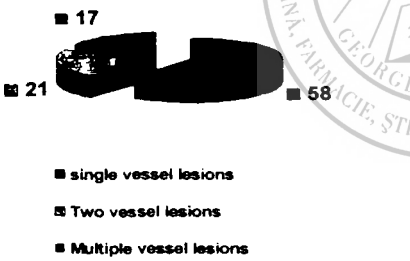

Figure 3.

Table I.

Inverted T waves +/- depressed ST segment	101
Other EKG changes	64
Total	165

Electrocardiographic changes were not significantly correlated with the severity of the coronary injuries (Table II).

Physicians are often faced with the problem of evaluating a patient with new-onset chest pain. Since angina of recent onset has been included under the rubric of unstable angina, the tendency in recent years has been to perform cardiac catheterization early. A comprehensive approach to diagnosis and medical management of angina pectoris is an integral part of the daily responsibilities of health care professionals.

Table II.

Multiple vessel disease		21
Normal resting EKG		4
Minor EKG changes		1
Major EKG changes (15)	Inverted T waves	6
	ST segment depression	1
	ST segment depression and inverted T waves	7
	ST segment elevation	-
	Conduction disturbances	1
Conduction disturbances		1

Approximately 30% of patients with chest pain referred for cardiac catheterization have normal or minimal atherosclerosis of coronary arteries. A subset of these patients demonstrates reduced coronary flow reserve. ECG is useful for evaluating persons with angina pectoris; however, findings are variable among patients. Approximately 50% of patients with angina pectoris have normal findings after a resting ECG. During an attack of angina pectoris, 50% of patients with normal findings after resting ECG show abnormalities. A 1mm or greater depression of the ST segment below the baseline, measured 80milliseconds from the J point, is the most characteristic change. Reversible ST-segment elevation occurs with Prinzmetal angina. Some patients with coronary artery disease may show pseudonormalization of the resting ECG ST-T–wave abnormalities during episodes of chest pain. Thus, the ECG changes are inconstant and unspecific, and their interpretation can only be made in clinical context.[1,4] Generally, the evaluation of ECG changes in unstable angina requires serial tracings or continuous monitoring, considering that the changes may vary from day to day or even during the same day.[5]

Coronary angiography remains the criterion standard for diagnosing coronary artery disease and is the primary method used to help delineate coronary anatomy. In addition to defining the site, severity, and morphology of lesions, coronary angiography helps provide a qualitative assessment of coronary blood flow and helps identify collateral vessels.

CONCLUSIONS

1. Patients with new-onset angina have an unusually large incidence of single vessel coronary artery disease.
2. The left anterior descending coronary artery is predominantly involved.
3. A significant number of patients with new-onset angina pectoris present severe coronary stenoses and multiple vessel lesions.
4. Changes in ECG traces do not correlate with the severity of coronary lesions.

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Apoptotic pathways of therapeutic potential in triple negative breast cancer

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Apoptosis plays an important role in embryogenesis, tissular homeostasis and cellular response to stimuli, therefore intensification of apoptosis is involved in pathogenesis of various ischemic, degenerative and immune imbalances. Triple negative (TN) breast cancer (negative for expression of estrogen and progesterone receptors (ER,PR) and HER2/neu protein) is a subtype of breast cancer with unique clinical and biologic profile, and usually with aggressive behaviour.

In our study, we investigated TRAIL and p53 immunostaining in the high-risk group of breast cancer with the (TN) phenotype that does not benefit from specific therapy that targets these proteins. Identifying additional immunohistochemical markers to refine prognostic in TN breast cancers is very much needed.

The aim of our study was to establish a correlation between TRIAL and p 53 imunostaining patterns, as biomarkers of apoptotic pathways, in an attempt of a better understanding of the biology of this special group of highly aggressive tumors.

***Key words**: triple negative breast cancer, TRAIL, p53, apoptosis*

Malignant breast cancers are in the majority of statistical studies on the 1st place in frequency and the 2nd leading cause of mortality in women. Despite progress in early diagnosis and genetic testing, there are many cases of recurrences and metastasis.

Breast cancer that do not express estrogen receptor (ER), progesterone receptor (PgR) or have no amplification of erythroblast bacteria oncogen homology 2/herstatin (erbB-2/HER2) are called triple-negative tumors. This phenotype is proven to have a poor prognosis. The identification of additional tumor markers might allow the selection of patients at different risk of tumor progression and death.

The originality of this study is the concomitant examination of TRAIL (Tumor necrosis factor-related apoptosis-inducing ligand) and p 53, the published data in the speciality literature on this subject being scant.

MATERIAL AND METHOD

For our study we selected, utilizing the documentation system SIGePMo (Institute Victor Babes, Bucharest, Romania), a list of 15 females patients with breast cancer, out of 162 consecutive breast tumors.

Sections of 5 microns were cut from formalin fixed, paraffin embedded tissue samples, deparaffinized in xylene and rehydrated through graded ethanol. Antigen retrieval was performed incubating the slides in 1 mM EDTA (pH 8.0) for 60 min, followed by blocking of the endogenous peroxidase activity in 3% H2O2 solution (10 min at room temperature) and incubated in blocking buffer (5% normal horse serum in PBS-0.05%) for 30 min at room temperature. Samples were incubated in appropriate antibody dilution (1:50, Novocastra in both cases) in blocking buffer overnight at 4°C. After being washed in PBS, slides were incubated with the peroxidase-conjugated secondary antibody (DAKO Envision system), for 30 min at room temperature. Peroxidase acitivity was detected with 3,3'-DAB as chromogen counterstained with hematoxylin. Mounted slides were examined by light microscopy.

Counting and scoring: immunoreactivity of TRAIL was assessed using a three-grade system, where 0 represented the absence of staining; 1 - minimal and variable staining and 2 - uniform, intense staining. Slides with score 1 and 2 were considered positive. For the p53 immunostaining we considered positive a percent of positive tumor cell over 10.

RESULTS AND DISSCUSIONS

The 15 patients included in this study were diagnosed with breast cancer. Specimens were obtained from total mastectomies. The patients were aged between 19 and 31 years old (mean 27.7).

We evaluated the tumoral histological type and grade (1-3), quantifing the tubule formation, nuclear and mitotic grades (Scarff–Bloom–Richardson grading system). All cases in our study were ductal carcinoma (Figure 1), 10 of them (66.66%) showing invasion, one (6.7%) intraductal with cribriform microlumina with irregular and scalloped contours (Figure 2) and 4 (26.66%) with both invasive and intraductal areas.

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Figure 1. Ductal invasive carcinoma HE, 200x

Figure 2. Ductal carcinoma I situ – HE, 200x

Nine cases were grade 3 (60 %), five cases grade 2 (33.33%) and one case grade 1 (6.7 %). Most cases (86. 66%) were circumscribed, with minimal stromal reaction. We also encountered apoptotic cells in all cases, areas of tumor necrosis with geographic distribution in 11 cases (73. 33%) and lymphoplasmacytic infiltrate in the stroma in 14 cases (93.33%). The number of invaded lymph nodes varied in each cases, ranging from 0 to 12: N0 – 4 cases (26.66%), N1 - 7 cases (46.66%). In 4 cases there were no informations on the lymph nodes (Nx). We did not detect the presence of angiolymphatic invasion in the breast tissue immediately adjacent to the tumor.

Slides with staining for hormonal receptors of all cases were reviewed. All selected cases were triple negative breast cancers (ER, PR, and Her2/neu). We considered as being negatives for hormone receptors slides with less than 5% or absent immunostaining for tumoral cells. The reactivity for Her2/Neu immunostaining was considered negative in slides showing score 0 and 1+ (> 10% of cells showing weak and incomplete membrane staining).

Six cases (40%) showed absence of staining for TRAIL. The positive cases (60%) showed cytoplasmic immunostaining in the tumoral cels with no nuclear or stromal staining. The nine positive cases were 33.33% score 1 (figure 3) and 26.66% score 2 (figure 4). Eleven cases were positive for p53, with a percentage of positive tumoral cells varying between 10 and 80% (figure 5).

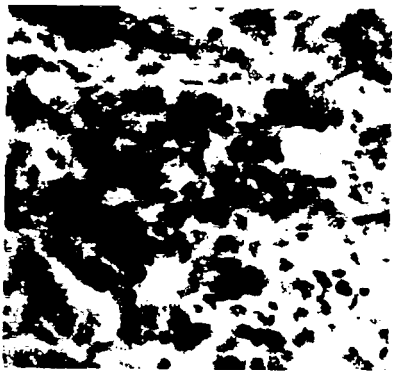

Figure 3. TRAIL positive in tumoral cells (score 1), 400x

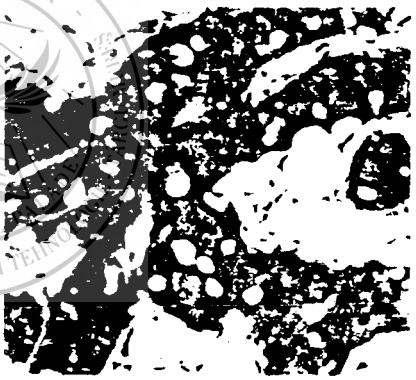

Figure 4. TRAIL positive in tumoral cells (score 2), 400x

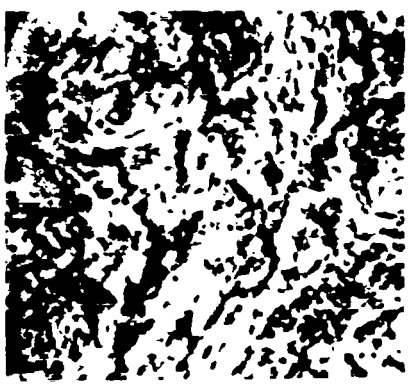

Figure 5. p53 positive in 80% of tumoral cells, 200x

Eight cases (53.33%) were both positive for TRAIL and p53, four cases (26.66%) were both negative for p53 and TRAIL and in 3 cases (20%) p53 was positive and TRAIL was negative. The direct correlation between 53 and TRAIL immunostaining was proved by calculating the Pearson correlation coefficient, r=0.64, and double sided p =0.009.

Approximately 15-20% of patients with breast cancer constitute a distinct subset by not expressing therapy targeted biological markers: ER, PR or the HER2/neu. These tumors tend to be more agressive, a better understanding of their biological processes being needed.

Apoptosis is an important mechanism of cell death and it may be a factor in tumor necrosis. In our study we identified apoptotic cells characterised by condensation of chromatin and cytoplasm and fragmentation and condensation of the nuclei on the histological sections in all cases. The mechanisms of apoptosis are essentially the same, whether induced by genetic signals or through external initiators. Genic aberrations that lead to the decrease or abolition of apoptosis promote tumorigenesis and resitance of cancerous cells at various genotoxic agents.

The proteasome is an enzymatic complex with a key role in degrading proteins involved in apoptosis into peptides and biological active molecules. [renx Because these defrading pathways are essential for cellular proliferation, especially in tumoral cells, a potential antitumoral therapy is represented by the proteasome inhibitors like PS-341 (Bortezomib, Velcade).[5,6,13] PS-341 has been shown to sensitize the tumoral cell to a pro- apoptotic molecule (TRAIL).[9]

Tumor necrosis factor-related apoptosis-inducing ligand - TRAIL, is a type 2 membrane protein of the TNF superfamily that selectively induces apoptosis of tumor cells but not of most normal cells.[8,10] TRAIL is regarded as a potential anticancer agent, but a considerable numbers of cancer cells, especially some highly malignant tumors, are resistant to apoptosis induction by TRAIL.[3,4,17] Coadministration of TRAIL with chemotherapy drugs significantly increases the degree of apoptotic cell death and drug sensitivity.[12]

In the study Rahman M et al[10], on triple negative breast cancers, upon treatment with TRAIL protein, 8 of 11 cell lines in the panel are sensitive to TRAIL-mediated apoptosis, showing a 10-fold increase in apoptosis as evidenced by an increase in the amount of fragmented DNA following cell lysis and 3 of the cell lines showed a three-fold increase in apoptosis. Increased expression of TRAIL has been observed in other human cancers: pancreas cancer[4] and non-small cell lung cancer tumors.[14]

Overexpression of mutant p53 is more likely to occur in patients with large primary, ER and PR-negative, aneuploid, or high S-phase tumors.[1] P53 positivity in triple-negative breast cancer is characterised by poor survival, prompting aggressive or alternative treatment.[2] In our cases the majority of cases (73.33%) of cases were positive or p53.

CONCLUSIONS

Modern therapies of malignant tumors have become more and more targeted, creating the need of new therapeutical technologies. A better understanding of molecular mechanisms of tumour growth and progression will allow not only the use of new molecular targets but also the interaction with various pathways of intra and extracellular signaling. As described in literature[10], the study of the emerging pathways of cell death downstream of the p53 tumor suppressor and the TRAIL death-inducing ligand opens ways to improve therapeutic design in cancer.

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Upper digestive endoscopic findings in asymptomatic patients taking low-dose aspirin

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The use of low-dose aspirin for primary or secondary prevention of cardiovascular disease increase the risk for symptomatic or complicated upper gastrointestinal tract ulcers by 2- to 4- fold.[3] The aim of our study was to identify the endoscopic changes occurred in chronic low-dose aspirin consumers free of dyspeptic symptoms. We enrolled 76 patients without dyspeptic symptoms, taking long term low-dose aspirin. They were questioned about their medical history and were evaluated by esophagogastroduodenoscopy. About 70% of patients presented various mucosal changes and about one thirty of them were identified with gastric or duodenal ulcers. The patients with positive endoscopy presented more frequent association of chronic disease, taking more drugs, were more frequent drinker, but the difference did not reach significance, except the smoking habits which was statistical significant more prevalent in patients with mucosal changes comparing with the patients group with negative endoscopy. Despite the absence of the symptoms, low-dose aspirin consumption increase the risk for digestive mucosal lesions in elderly users who need gastroprotective strategies to prevent major complications (bleeding).

***Key words**: aspirin, gastropathy, endoscopy*

The use of ant platelet therapies continues to increase as a result of accumulation of evidence of benefits in both primary and secondary treatment strategies for cardiovascular disease. Low-dose aspirin, commonly defined as 75-325 mg/daily, is used routinely to prevent myocardial and cerebral vascular ischemia, although a reduction in mortality has not been documented.[1] The main factor limiting the use of aspirin is concern about the development of gastrointestinal events, especially gastrointestinal bleeding. Aspirin produces its antithrombotic effect via irreversible acetylating of serine in cyclooxygenase-1 (COX-1) in platelets. This abolishes production of thromboxane A_2 for the life of the platelet. Inhibition of the COX-1 pathway blocks production of prostaglandins that play an important protective role in the stomach by increasing mucosal blood flow and stimulating the synthesis and secretion of mucus and bicarbonate, as well as promoting epithelial proliferation. A major consequence of prostaglandin depletion is to create an environment that is conductive to peptic ulcer formation and serious gastrointestinal complications.[2] The average incidence of upper gastrointestinal complications would be close to 2-3 cases per 1000 person-years for aspirin users, the relative risk of major gastrointestinal bleeding with low-dose aspirin in a meta-analysis of placebo-controlled trials of cardiovascular protection being 2.7 (95% CI:1.61-2.66).[3] Factors that may increase the risk of gastrointestinal bleeding include the age, prior history of ulcers or gastrointestinal events, corticosteroid use, anticoagulant therapy and addition of a non-aspirin non-steroidal anti-inflammatory drug.[3] The net benefit of aspirin depends on the initial risk for coronary heart disease and gastrointestinal bleeding. Thus, decision about aspirin therapy should consider the overall risks for coronary heart disease and gastrointestinal bleeding.[1] The aim of the present study was to assess the prevalence of upper gastrointestinal mucosal changes in asymptomatic patient taking long time low-dose aspirin.

MATERIAL AND METHOD

A prospective open design study was used. Consecutive patients admitted in III[rd] Medical Clinic in Târgu-Mureș or attending the outpatients department, accepting an esophagogastroduodenoendoscopy (EGD) were enrolled. All the patients included had a clear cardiovascular indication for antiplatelet therapy and they had a medical history of more than 1 month of regular low-dose aspirin 75-250mg/day. Exclusion criteria were: patients with dyspeptic symptoms (upper abdominal pain, heart burn), patients with history for ulcer disease or drug induced gastropathy, severe chronic disease that prevented endoscopy. We recorded details of patients' demography, medical history, symptoms, particularly cardiac and gastrointestinal complaints, clinical observations, drug intake, alcohol and smoking habits.

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Antral and body biopsies were taken routinely for Helicobacter pylori (H. pylori) status and also, were taken supplementary biopsies from other incidental lesions (polyps, ulcers, Barrett esophagus appearances). Hematoxylin-eosin (H&E)-stained gastric mucosal specimens were used to evaluate the extent of inflammation and atrophy. All patients signed an informed consent. The GraphPad program was used to analyze the data. Statistical significance was defined as $p<0.05$.

Table I. Modified Lanza score for drug induced endoscopic findings (Lanza FL et al, 1998)

Score	Endoscopic findings
0	Normal mucosa
1	1 to 3 erosions or submucosal hemorrhages
2	4 to 10 erosions or submucosal hemorrhages
3	>10 erosions or submucosal hemorrhages
4	Ulcer or diffuse submucosal hemorrhages

RESULTS AND DISCUSSIONS

A total number of 76 low-dose aspirin consumers, 41 men and 35 women, with mean age 65.89 years, free of dyspeptic symptoms, met the including criteria and were enrolled. Endoscopic findings were classified according to the modified Lanza's scale (Table I).[4] Patients were divided into two groups: first group with negative EGD, 36 patients with normal appearance of gastroduodenal mucosa (modified Lanza score 0) and the second group, with positive EGD, 40 patients presenting various mucosal changes (modified Lanza score 1, 2, 3, 4). The endoscopic and histological findings are shown in table II.

In patient group with mucosal changes, 16 patients (40.0%) were included in Lanza 1 score, 7 patients (17.5%) in Lanza 2, 5 patients (12.5%) in Lanza 3 and 12 patients (30.0%) in Lanza 4 score. The predilection of the stomach to ulcers or erosions has been described, and it is typical for non-steroidal anti-inflammatory gastropathy, including aspirin and was confirmed in our study. Regarding the H. pylori status, a total of 25 patients (32.89%) from both groups was positive in biopsy stains. Histological examinations shown the presence of intestinal metaplasia and/or gastric mucosal atrophy in 37 patients (48.68%): 8 (22.2%) in the group with negative EGD and 29 (72.5%) in the group with positive EGD. The difference was statistical significant between the two group in this point of view ($p<0.05$).

The demographic and clinical data are shown in Table III. The low-dose aspirin represent 75mg/day in 66 patients, 100mg/day in 3 patients, and 250mg/day in 7 patients. The mean daily dose aspirin was in our study 90.78mg/day, lower than 129,34 mg/day which results in other western studies.[5] Comparing the two groups of patients, study not yielded statistical difference regarding the age, sex, alcohol intake, chronic disease or other drugs used. Nevertheless, patients with mucosal lesions presented more frequent chronic diseases, taken more non-steroidal anti-inflammatory drugs and/or oral anticoagulant, were more frequent drinkers, which can increase the risk for major gastrointestinal complications and these results are comparable with others obtained in larger studies.[6] Only smoker status was statistical significant more frequent in our patients with gastroduodenal lesions, comparing with patients with negative EGD.

In our study the mean age of the asymptomatic patients taking long time low-dose aspirin was about 65 years, in other western studies, these age being around 70 years[5], due probably to the difference in general population characteristics. Unlike results of studies among other non-steroidal anti-inflammatory drugs, case-control studies have consistently shown that H. pylori is an important risk factor for ulcer and ulcer bleeding in users of low-dose aspirin.[1] In our study the presence of H. pylori infection was more frequent in patients with positive EGD comparative with patients without lesions (37.7% vs. 27.5%), but the difference was not statistical significant. Thus, the H. pylori infection was not the main etiological factor in these lesions. The presence of premalignant lesions (intestinal metaplasia and gastric mucosal atrophy), which are known being induced by H. pylori infection, are statistical significant more frequent in patients with Lanza score above 0. This observation cannot completely exclude the role of H. pylori infection in aspirin induced gastropathy, possible due to the high prevalence of infection in our region. Several studies document that the addition of a non-aspirin non-steroidal anti-inflammatory or oral anticoagulant drug to low-dose aspirin significantly increase the risk of developing upper gastrointestinal bleeding-by about

Tabel II. The endoscopic and histologic findings in study group patients

	Lanza 0	Lanza 1	Lanza 2	Lanza 3	Lanza 4	p
	N=36 %	N=40 %				
Gastric lesions		16(40.0)	7(17.5)	5(12.5)	12(30.0)	
Duodenal lesions		13 (32.5)	7 (17.5)	3 (7.5)	7 (17.5)	
Concomitant lesions		2 (5.0)	0	1 (6.2)	3 (7.5)	
H pylori positive	10 (27.7)	1 (6.2)	0	1 (6.2)	2 (5.0)	NS
Intestinal metaplasia/ gastric atrophy	8 (22.2)	15 (37.5)				
		7 (17.5)	3 (7.5)	1 (6.2)	4 (10.0)	P<0.0001
		29 (72.5)				
		10 (25.0)	6 (15.0)	4 (10.0)	9 (22.5)	

Table III. The demographical and clinical data in study group patients

	Lanza score 0 (n=36)		Lanza score 1,2,3,4 (n=40)		
	n	%	n	%	p
Mean age	64.89		66.90		NS
Male	14	38.88	30	75.00	
Aspirin					
75mg/day	30	83.33	36	90.00	NS
100mg/day	2	5.55	1	2.50	NS
250mg/day	4	11.11	3	7.50	NS
Smokers	7	19.44	23	57.50	p=0.0009
Current smoker	6	16.66	6	15.00	NS
Former smoker	1	2.77	17	42.50	p=0.086
Non-smoker	29	80.55	17	42.50	NS
Alcohol intake*					
Drinker	5	13.88	11	27.50	NS
Occasionally drinker	16	44.44	17	42.50	NS
Non-drinker	15	41.66	12	30.00	NS
Chronic disease					
Arterial hypertension	35	97.22	39	97.50	NS
Ischemic heart disease	9	25.00	13	32.50	NS
Congestive heart failure	4	11.11	6	15.00	NS
Stroke	7	19.44	8	20.00	NS
Respiratory disease	6	16.66	13	32.50	NS
Diabetes mellitus	7	19.44	7	17.50	NS
Drug used					
Nitrate	12	33.33	21	52.50	NS
NSAID**	20	55.55	26	65.00	NS
Within last 7 days	5	13.88	7	17.50	NS
Within last 30 days	15	41.66	19	47.00	NS
Oral anticoagulant	4	11.11	11	37.50	NS

Note: *Drinker>10 drinks/week (1 drink=14 grams pure alcohol). Occasionally drinker<10 drinks/month (The Alcohol Use Disorder Test, AUDIT)**NSAID = non-steroidal anti-inflammatory drug

two- to fourfold.[2,3] Our study not yielded statistical significant differences in patients using low-dose aspirin concurrent with other non-steroidal anti-inflammatory drugs or anticoagulants, but gastric and/or duodenal lesions were more severe in these patients.

CONCLUSIONS

Positive endoscope finding in more than 50% of asymptomatic patients taking low-dose aspirin and one thirty of these presenting ulcer, should alert the physicians for the "silent" gastrointestinal risks of this medication. The absence of symptoms may be dangerous, because the lack of alert signs for severe complications (bleeding, perforation) which can be fatal in elderly population. More than one thirty of patients with mucosal lesions was positive for H. pylori infection, and current evidence shown that eradication therapy in patients with a history of ulcer reduces the risk for gastrointestinal bleeding in low-aspirin users. Despite the absence of symptoms, more than a half of patients in our samples presented risk factors for gastrointestinal bleeding and they need gastroprotection therapy. Communication between cardiologists, gastroenterologists, and primary care physicians is critical to weigh the ischemic and bleeding risk in an individual patient who needs ant platelet therapy but who is at risk for develops significant gastrointestinal bleeding.

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Prognostic evaluation of bone marrow infiltration in chronic lymphocytic leukemia

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The most frequently used staging systems in chronic lymphocytic leukemia are the Rai and Binet stages. But these systems not always predict correctly the clinical evolution and the prognostic of the disease. In these systems the bone marrow biopsy pattern is not included, though it is an important prognostic parameter. The bone marrow pattern types are: interstitial, nodular, mixed and diffuse, the latter two are with the worst prognostic. In our study we estimated the survival of the patients with diffuse and non-diffuse patterns.
We observed a group of 212 patients, with the age between 38-86 years.
In a number of 44 patients, we evaluated the bone marrow pattern, and we found: 24 patients with diffuse pattern and 20 patients with non-diffuse pattern. The median survival of the first group was 18 months and 96 months at the second group. 18 patients were in stage Binet C, 16 in stage Binet B and 10 in stage Binet A.
The bone marrow pattern is a tumor mass indicator and brings supplementary information about prognostic.
***Key words**: leukemia, lymphocyt, diffuse, prognostic*

Chronic lymphocytic leukemia is a primitive disease of the lymphoid tissues, characterized by the malignant proliferation and accumulation of a small lymphocytes clone, mature appearing but immunologic incompetent.

Chronic lymphocytic leukemia has a extreme variable evaluation. Median survival of these patients is about 10 years, with large variation between a normal survival for a health population of the same age and a very short survival.

The most known prognostic factors are the two major staging systems, Rai and Binet:

-Stage Rai 0, Binet A- low risk, survival over 10 years;

-Stage Rai I/II, Binet B- intermediate risk, survival between 5 and 7 years;

-Stage Rai III/IV, Binet C- high risk, survival between 1-3 years.

But in these staging systems, it isn't included the bone marrow biopsy pattern, a very important prognostic marker in chronic lymphocytic leukemia.

The patterns of marrow infiltration are established from marrow histology, which can reveal four patterns:

I. Interstitial - infiltration with lymphocytes between normal marrow cells;

II. Nodular- nodules made up mature lymphocytes appear, these nodules are greater than normal lympoid follicles;

III. Mixed- a combination of interstitial and nodular patterns;

IV. Diffuse- diffuse lympoid infiltration with massive replacement of normal hemopoietic tissue as well as fat cells.

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A non-diffuse replacement of the marrow (interstitial, nodular and mixed), is associated with the early stages and a good prognosis. A diffuse replacement of the marrow is associated with a worse prognosis and the advanced stages.

The aim of the study was the prognostic evaluation at the bone marrow infiltration and his role in survival and disease progression.

MATERIALS AND METHODS

We observed a group of 212 patients, with chronic lymphocytic leukemia, who were diagnosed and treated in our clinic between the years 1989-2008.

The diagnostic criteria for CLL were those usually recommended by the International Workshop on CLL: 1) more than 10.000/mmł lymphocytes in peripheral blood, which remain four weeks, or 2) more than 5.000/mmł monoclonal lymphocytes, evaluated from flow cytometric analyses, 3) more than 30% marrow infiltration.

The B-cell CLL phenotype are CD5 positive, CD19 positive, CD23 positive, CD22 week positive, SmIg week positive, FMC7 negative.

In 44 patients with CLL, bone marrow biopsy was carried out as part of their initial staging procedure. We divided our lot in two groups: non-diffuse pattern and diffuse pattern.

At these patients, we analysed more charactersistics:

a) Patients' age

b) The bone marrow biopsy pattern and the median survival depending on bone marrow pattern

c) The absolute lymphocyte count at diagnosis and the marrow infiltration with leukemic cells, in diffuse group, comparative with non-diffuse group

d) The hemoglobin count at diagnosis in the each two groups

e) The clinical stage of the disease and median survival depending on the stage.

RESULTS

The mean age of our lot was 65,6 years, with the age between 38 and 86 years. Ten patients had the age less than 60 years and 34 patients more than 60 years.

The lot was made by 28 males and 16 females.

The patterns of marrow infiltration were: 24 patients with diffuse pattern, 7 patients with interstitial pattern, 9 patients with mixed pattern and 4 patients with nodular pattern (Image 1).

We had 54,54% patients with diffuse pattern and 45,45% with non-diffuse pattern (Image 2).

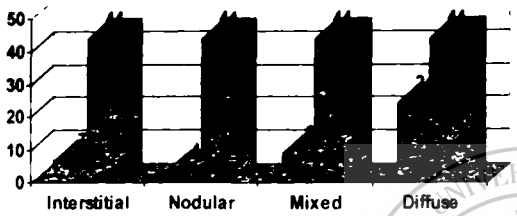

Image 1. The lot distribution depending on bone marrow biopsy patterns

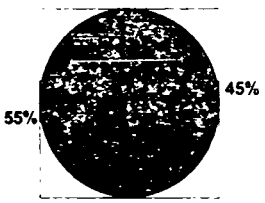

Image 2. The bone marrow biopsy pattern in percentage

Depending on the Binet stage of the disease we found 18 patients in stage Binet C, 16 patients in stage Binet B and 10 patients in stage Binet A (Image 3).

Median survival of the patients with diffuse pattern was 18 months and 96 months for the patients with non-diffuse pattern.(p<0,05) (Image 4).

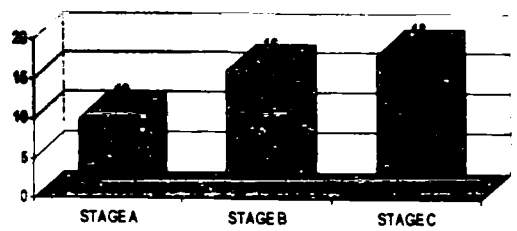

Image 3. The lot distribution depending on stage

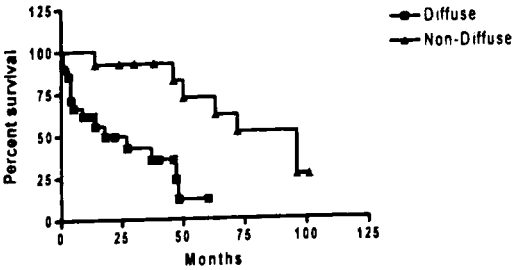

Image 4. The median survival depending on bone marrow biopsy pattern

Median survival of the patients in the stage Binet C was 14 months, versus 50 months for the patients in stage Binet B and 96 months in stage Binet A.(p<0,05) (Image 5).

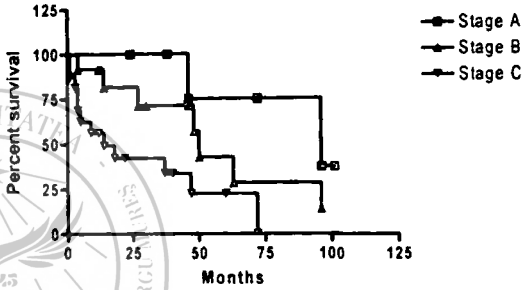

Image 5. The median survival depending on Binet stage

In the group with pattern diffuse we observed at the 18 (75%) patients, a lymphocyte count more than 30.000/ul, versus 8 (40%) patients with the same lymphocyte count in the other group with non-diffuse pattern.

We found the hemoglobin at the diagnosis, less than 12 g/dl, at 18 (75%) patients in the first group, versus 7 (35%) in the second group.

We found the marrow infiltration with lymphocytes more than 80%, at the 13 (54,16%) patients in the group with diffuse pattern, versus 6 (30%) patients in the group with non-diffuse pattern (Table I).

Tabel I. The correlation between bone marrow biopsy pattern and others prognostic markers

he criterion	NON-DIFFUSE	DIFFUSE
Lymphocytosis>30.000/ul	8 (40%)	18 (75%)
Hemoglobin<12g/dl	7 (35%)	18 (75%)
Marrow-Ly>80%	6 (30%)	13 (54,16%)

In our study we observed that in the first group with diffuse pattern the patients are in advanced stages, Binet B and C. Therefore 13 (54,16%) patients are in stage Binet C, versus 5 (25%) patients by the group with non-diffuse pattern.

In the lot with diffuse pattern we had 9 (37,5%) patients in stage Binet B and 2 (8,33%) patients in early

stage Binet A, versus the group with non-diffuse pattern where we found 7 (35%) patients in stage B and 8 (40%) in stage A (Image 6).

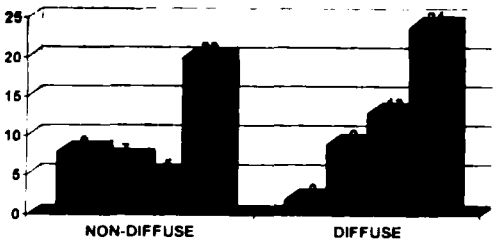

Image 6. The patients distribution depending on Binet stage

DISCUSSIONS

Up the time many studies are tried to find a correlation between the different prognostic markers and the survival of the patients with chronic lymphocytic leukemia.

Though the clinical stage are the most simple and widely used prognostic marker, exist anothers clinical and laboratory factors, which tried to estimate correct the prognostic of this disease.

The most controversial prognostic parameter are bone marrow biopsy pattern.

The bone marrow pattern and his prognostic importance was the subject at the many studies and their conclusion is that the patients with diffuse pattern have the worst prognostic.[8]

Some studies have been demonstred that diffuse pattern is an independent prognostic marker and much more is a value prognostic marker in disease evolution.[8]

Our study demonstred the correlation between diffuse pattern and unfavorable clinical evolution. And also we observed the correlation between diffuse pattern and advanced stage of the disease.

The studies have been reported a shorter survival in the group with diffuse pattern and that appeared to be a stronger predictor of survival than hepatosplenomegaly, lymphadenopathy, anemia and trombocytopenia.[12]

Also the studies was observed that the patients with diffuse pattern have the indication to begin the treatment even in the early stages.[14]

However, some reports have suggested that bone marrow biopsy pattern did not appear to have a predictive value beyond that provided by the clinical stage.[14]

What about the stage, in the present study, was demonstred a good survival in the stage Binet A and B, and a short survival in the stage Binet C.[14]

In our study, we observed that the patients with diffuse pattern to associate with other worse prognostic markers, according to The French Cooperative Group on Chronic lymphocytic Leukemia: lymphocytes count more than 30000/ul at diagnosis, hemoglobin less than 12g/dl at diagnosis and marrow infiltration more than 80% at diagnosis.[9]

Also we observed that in diffuse group the patients are in advanced stages, comparing with non-diffuse group, where only L patients are in stage Binet C.[12]

These patients, in the stage C, have anyway a worse prognostic, because is a advanced stage.

CONCLUSIONS

In the present group we fallowed the bone marrow biopsy patterns at the patients with chronic lymphocytic leukemia and we tried to make a correlation with others prognostic factors, demonstrating the worst prognostic at the group with diffuse pattern.

In our study we observed that almost patients with diffuse pattern are in stage Binet C, the stage with unfavorable prognostic. Therefore remain in debate if the prognostic is get by bone marrow biopsy pattern or by the advanced stage.

These parameters, doesn't differentiate, the patients in early stages which have others prognostic factors, like citogenetic and molecular anomaly, who have unfavorable prognostic indifferent by stage and bone marrow biopsy pattern.

Therefore it's important to estimate how many prognostic markers, for to employ correct the patients in a group with favorable prognostic or another with worse prognostic.

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The efficiency of treatment with iloprost (ilomedin) and sildenafil (viagra) in neonatal primary (idiopathic) pulmonary hypertension

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Introduction: Persistent pulmonary hypertension of the newborn is a syndrome characterized by elevated pulmonary vascular resistance that causes refractory hypoxemia and right-to-left extrapulmonary shunting of blood through patent ductus arteriosus and foramen ovale. The main causes of primary pulmonary hypertension in neonate are: excessive muscularization of pulmonary arterioles and alterations in vasoactive mediator levels. Case report: the authors present a case of idiopathic PPHN in a term newborn treated with i.v. Iloprost (prostacyclin analogue – Ilomedin 20) and oral sildenafil (phosphodiesteraze-5 inhibitor - Viagra), in a situation where the standard treatment - inhaled nitric oxide (iNO), high-frequency ventilation (HFV), and extracorporeal membrane oxygenation (ECMO) was not available in our unit. The therapeutic protocol was: Iloprost i.v. 1-2ng/kg/min, and oral sildenafil 1-2mg/kg/dose q 6 hours. Results: Iloprost administration showed significant improvement of alveolar-arterial oxygen gradient (A-a)O_2 and arterial oxigenation (PaO_2 and SaO_2) within the first 16 hours of administration, augmented by oral sildenafil administration. Iloprost was withdrawn after 96 hours, sildenafil administration was continued for 9 days with decreasing doses. Serial echocardiograms showed decreasing pressures in the pulmonary artery to normal values. Conclusions: the combination of i.v. Iloprost and oral sildenafil showed important pulmonary vasodilatator effect, with high clinical efficacy in this case. Further data are needed to determine effects on larger groups of newborns with PPH.
***Key words**: Iloprost, sildenafil, idiopathic PPHN*

Persistent pulmonary hypertension of the newborn (PPHN) is a syndrome characterized by elevated pulmonary vascular resistance (PVR) that causes hypoxemia and right-to-left extrapulmonary shunting of blood. Inadequate pulmonary perfusion develop refractory hypoxemia, respiratory distress, and acidosis. It affects term or postterm (near-term) infants.

Pulmonary hypertension can be primary (idiopathic) or secondary to pulmonary parenchymal disease, pulmonary hypoplasia, polycythemia, hypoglycemia, sepsis or maternal ingestion of prostaglandin inhibitors.[1,2,5] The primary-idiopathic term describes the condition when no cause for the PPHN can be found, so it is used as a synonim for unexplained pulmonary hypertension. In these cases, the syndrome is the result of an excessive muscularization of pulmonary arterioles or the result of alterations in vasoactive mediator levels.[3,5]

The pathophysiology of PPHN is not only dependent on a deficiency in the vasodilator modulators (O_2, NO, PG), but may also result from an excess of vasoconstrictor modulators (hypoxia, endothelin-1, leukotriene, thromboxane A_2). It is possible that genetic factors may play a role in developing PPHN. Many authors sustain that PPHN is associated with low-grade chronic intrauterine hypoxia, so unrecognized asphyxia may account for many cases labeled as „idiophatic".[5,6,8,10,12,13,14]

Symptoms and signs include: progressing respiratory distress with tachypnea, retractions, severe cyanosis or desaturation unresponsive to supplemental O_2 and acidosis. It can mimic the symptoms of cyanotic congenital heart disease. Cardiac examination: systolic murmur secondary to tricuspid regurgitation. The patient may have evidence of poor cardiac function and perfusion. The idiopathic PPHN is associated with a normal chest radiograph and no parenchymal lung disease and it presents like pure vascular disease. Diagnosis is by history, examination, chest x-ray, and response to O_2. The clinical diagnosis of PPHN is considered when there is hypoxemia refractory to oxygen therapy: PaO_2 < 55 mmHg despite FiO_2 of 100% associated with a preductal to postductal oxygen gradient greater than 20 mm Hg.[2,5,12]

The diagnosis is confirmed by echocardiogram: the presence of elevated pressures in the pulmonary artery using Doppler investigation and tricuspid regurgitation.

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Advanced management of PPHN include: high frequency ventilation (HFV), inhaled nitric oxide (iNO), extracorporeal membrane oxygenation (ECMO), pulmonary vasodilatators: phosphodiesterase inhibitors (sildenafil), prostacyclin analogues (iloprost). Some of these modalities are expensive and not available in all units.[3,4,7,9,11,14]

CASE REPORT

The authors present a non-ventilated case of idiopathic PPHN where we used i.v. Ilomedin combined with oral sildenafil as a therapeutic option in the treatment of pulmonary hypertension because the standard of practice (iNO, HFV and ECMO) was not available.

Iloprost is a syntethic analog of prostaglandin I_2 (PGI_2 - prostacyclin). PGI_2 is one of the major endogenous vasodilators in the lung. It is normally produced by the lung when lung vessels are in constricted state, thereby producing relaxation. The prostacyclins act through an increase in the level of cyclic adenosine monophosphat (cAMP).[15]

Ilomedin is a stable solution, the half-time is 20-30 minutes, and administered intravenously has linear pharmacokinetics over the dose range of 1-3 ng/kg/min. The plasma concentration of iloprost falls very quickly after the end of the infusion because of the high rate of metabolism. Ilomedin is metabolised principally in the liver; 80% of the iloprost metabolites are excreted via the kidneys and 20% with the bile. We used Ilomedin 20 – for intravenous use, 1 ml ampules, concentration: 20 µg/ml). Start dose: 1 ng/kgc/min.

Sildenafil is a phosphodiesterase-5 inhibitor (PDE 5) and acts by increasing cyclic guanosine monophosphat (cGMP) and cAMP. Sildenafil auguments and prolongs the effects of Iloprost.[4,9,11,15]

A 50-mg tablet of sildenafil (Viagra) was divided in doses of 5mg powder (each was diluted in distillated water for a final concentration of 2,5 mg/ml). The drug was given by orogastric tube. We administrated 2 mg/kg sildenafil every 6 hours in parallel with intravenous adinistration of Ilomedin.

It was followed:

1. Arterial blood gases from an arterial catheter (radial artery)

-partial pressure of oxygen (PaO_2)< 35 mmHg and FiO_2 requirement

-alveolar-arterial gradient (A-a): difference in the partial pressure of P_AO_2 - PaO_2

-PaO_2/FiO_2

Changes were measured 4 times a day, at 30 minutes after each dose of oral sildenafil combined with continuous perfusion with Iloprost in the first 96 hours of combined treatment, at 30 minutes after each dose of oral sildenafil as a monotherapy till the end of treatment (9 days).

2. Continuous pulseoximetry: oxygen saturation (SpO_2)

3. Arterial blood pressure (BP) every 3 hours.

RESULTS

-Birth date: 26 march 2008, gestational age: 41 weeks, birth weight: 4300g, LGA, lenght: 59 cm

-rupture of membranes: 6 hours before delivery

-Vaginal delivery, cranial presentation

-Apgar scores: 5/1 minute, 8/5 minutes, 9/10 minutes. Resuscitation in delivery room: permeabilization of respiratory airways, tactile stimulation, bag and mask ventilation for 1 minute, O_2 administration for 3 minutes. Blood gases from ombilical cord: pH 7,21, pCO_2 48 mmHg, pO_2 35 mmHg, BE -6,3.

-After birth (5 hours): cyanosis, tachypnea and respiratory distress (Silverman score 5), with frequent desaturations SpO_2 78-80 %, without SpO_2 gradient preductal (right hand)-postductal (left foot), cardiac examination revealed a III/6 grade systolic murmur.

-The echocardiogram showed evidence of right-to-left shunt via persistent foramen ovale, pulmonary artery pressures 73-74 mmHg, significant tricuspid regurgitation, no congenital abnormalities.

-Chest radiography: no parenchymal lung disease, clear, hyperlucent lung fields

-Complete blood count in the first 2 hours of life: L 23.300/mm^3, H 4.310.000, Hgb 17,2 g%, Htc 53,1 %, Platelets 214.000

-Complete blood count at 24 hours: L 16910/mm^3, Hgb 15,7 g%, Htc 45,1 %, Platelets 197.000, Meta 4%, NS 31 %, Sg 43 %, Eo 1 %, Ba 2 %, Mo 7%, Li 12 %, immature/total neutrophile index I/T: 0,44

-Culture of gastric aspirate: negative, hemoculture: negative

The diagnosis was idiopathic PPHN. We exclude cardiac and pulmonary diseases.

Therapeutic management

-incubator, total parenteral nutrition via umbilical vein catheter, O_2 administration via oxygen hood, FiO_2 60%, antibiotherapy: iv Ampiciline+Netromycine

-Ilomedin 20 starting dose: 1 ng/kgc/min iv

-Sildenafil (Viagra) starting dose: 2 mg/kg orally

-Minimal handling and stimulation

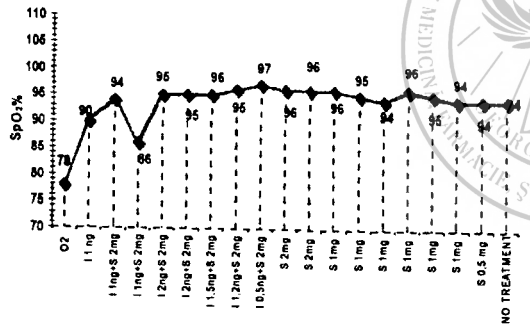
The variations of PaO_2, SaO_2, $(A-a)O_2$, PaO_2/FiO_2, FiO_2, related to the used doses of i.v. Ilomedin and oral sildenafil are shown in table I.

We noticed significant increase of SaO_2 in the first 30 minutes after i.v. administration of prostacyclin, but with the persistance of O_2 instability. Oral sildenafil was associated after 4 hours, but we needed dubling the starting of i.v. Iloprost dose after 12 hours because increasing the necessity of O_2 (FiO_2). Further maintenance of an optimal level of oxygenation was obtained after 6 doses of sildenafil (2mg/kg) in parallel with continuous infusion of Iloprost.

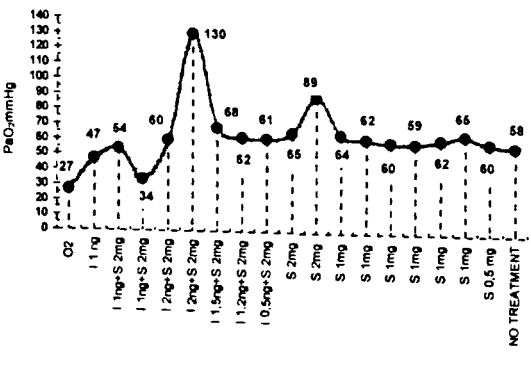
After 48 hours of combined therapy with i.v. Iloprost and oral sildebafil decrease of pulmonary hypertension confirmed by clinical signs, significant increase of PaO_2 and ultrasonographic findings permited gradual decrease of i.v. Iloprost doses, beeing withdrawn totally after 96 hours. Oral sildenafil

Table 1. The variations of PaO_2, SaO_2, $(A-a)O_2$, PaO_2/FiO_2, FiO_2, related to doses of i.v. Ilomedin and oral sildenafil

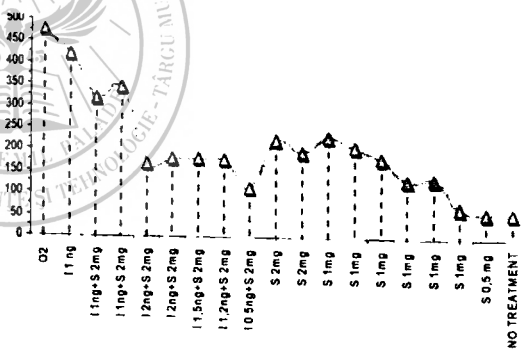
TREATMENT	PaO_2	FiO_2	(A-a)	PO_2/FiO_2	SaO_2
O_2 administration	26,6	80	475,7	0,33	78
Ilomedin 1ng/kg/min	47	70	421	0,67	90
Ilomedin1ng/kg/min + Viagra 2 mg/kg	54,3	60	318,2	0,90	94
Ilomedin1ng/kg/min + Viagra 2 mg/kg	33,5	75	344,5	0,44	86
Ilomedin ng/kg/min + Viagra 2 mg/kg	59,9	40	167,9	1,50	95
Ilomedin2ng/kg/min + Viagra 2 mg/kg	130,1	50	178,8	2,62	95
Ilomedin1,5ng/kg/min+Viagra2 mg/kg	68,4	50	180,2	2,7	95
Ilomedin1,2ng/kg/min+Viagra2 mg/kg	62,2	40	177,1	1,8	96
Ilomedin0,5ng/kg/min Viagra 2 mg/kg	61,0	30	112,5	2,03	97
Viagra 2 mg/kg	65,4	40	221,1	1,23	96
Viagra 2 mg/kg	88,8	40	193,2	1,68	96
Viagra 1 mg/kg	63,9	40	228,1	1,27	98
Viagra 1 mg/kg	61,8	40	204,5	1,41	96
Viagra 1 mg/kg	59,7	36	180,1	1,65	95
Viagra 1 mg/kg	59,2	30	130,4	1,97	94
Viagra 1 mg/kg	62,1	30	132,6	2,07	96
Viagra 1 mg/kg	65,4	21	70,8	3,11	95
Viagra 0,5 mg/kg	58,9	21	59,2	2,80	94
NO TREATMENT	58,2	21	58,4	2,77	94



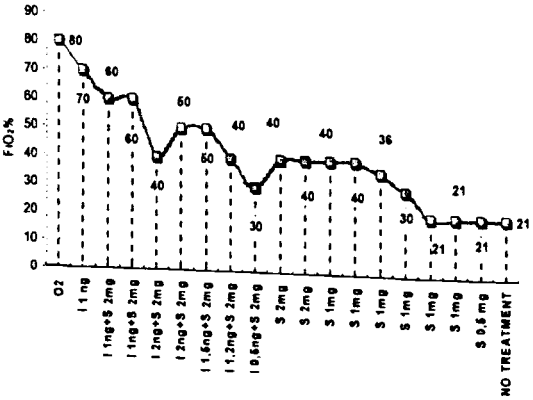
Graphic 1. $SpO_{2\%}$ variation



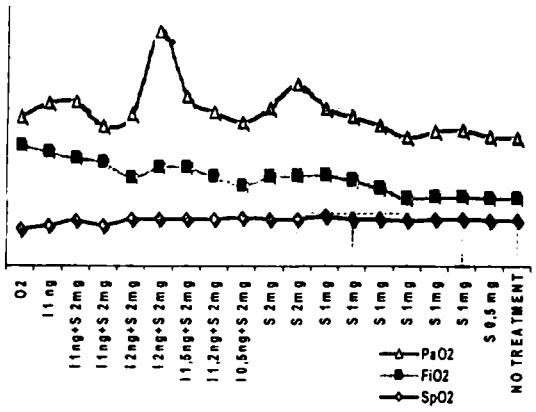
Graphic 2. PaO_2 variation



Graphic 3. O_2 (A-a) variation



Graphic 4. FiO_2% variation



Graphic 5. SpO_2, FiO_2, PaO_2 variation

administration needed to be continued 9 more days, 2 days in the same doses, 6 days with half doses (1 mg/kg/dose) and 1 day with 0,5 mg/kg/dose until arterial pulmonary pressure reached the normal values for age. Further evaluations showed no rebound of pulmonary hypertension.

After the 4th dose of oral sildenafil (2mg/kg) and 24 hours of continuous administration of Iloprost, alveolar-arterial gradient (A-a) started to decrease significantly.

After 24 hours of i.v. Ilomedin and first doses of oral Sildenafil (2mg/kg) it was possible to decrease FiO_2 to 50%, O_2 supplementation beeing withdrawn in 12 days.

FiO_2, PaO_2 and SpO_2 changes related to combined vasodilatators doses are showned in graphic 5.

CONCLUSIONS

1. Associated administration of prostacyclin and sildenafil determined a significant elevation of arterial oxygenation and improvement in clinical status.

2. Minimal side effects were noticed: mild cutaneus vasodilatation

3. Combined selective vasodilator treatment with i.v. Ilomedin and oral Sildenafil i this case of idiopathic PPHN proved its effectiveness in lack of standard therapy (HFV, iNO).

4. The newborn was included in our follow+up program and had good neurological outcome at the age of two months.

5. Further data is needed to determin efects on larger groups of newborns with PPPH.

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The long-term follow-up also verifies the great posttreatment predictive value of FDG-PET in Hodgkin lymphoma patients

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Background. Determining the viability of residual tumor masses is a great challenge after treatment of Hodgkin's lymphoma. FDG-PET may play a crucial role in this procedure.
Design and Methods. In this study, files of 128 Hodgkin's lymphoma patients, who were treated between January 1995 and February 2005 were reviewed, retrosepctively. Their median follow-up was 75.5 months from PET examination.
Results. The number of true-positive, true-negative, false-positive, false-negative subjects were 29, 83, 10, 6, respectively. Sensitivity of post-treatment FDG-PET was 83 %, specificity 93 %, positive predictive value 74 %, negative predictive value 93 %, and accuracy 88 %. According to the Mantel-Cox test, the difference between the event free survival of PET positive and negative cases is highly significant (p <0.0001). Our results in a large cohort of patients, in accordance with literature, clearly indicates that patients with negative FDG-PET results are unlikely to progress or relapse during a very long follow-up. The shortest remission time among PET negative patients was 33 month.
Conclusion. The most important observation is, that FDG-PET examination is able to prognosticate early relapse with 100 % NPV, but it is not so sensitive in the prediction of late relapses. PPV is not as high as NPV, so PET positive cases must be carefully analyzed, biopsy must be done when possible.
Running head: Posttreatment prognostic value of FDG-PET in HL
***Key words**: Posttreatment FDG-PET, Hodgkin lymphoma, prognosis, long-term follow up*

In the past few decades Hodgkin lymphoma (HL) has become a highly curable malignant disease. The problem of residual mass after induction therapy is often difficult in HL. Approximately two-third of HL patients present with residual masses after completion of their planned treatment, but only 20-25 % will finally relapse.[2] These masses may contain residual lymphoma, which needs further treatment or may represent fibrosis or necrotic tissue. The early diagnosis of relapse, progression or incomplete response is an important indication to start salvage therapy. If the tumor is easily accessible, the questionable lesion can be excised and histologically analyzed, whereas a deep tumor can only be accessed by open thoracic or abdominal surgery. In the 1990s, positron emission tomography (PET) scanning was recognized as a powerful tool in the diagnosis and staging of tumors, including lymphomas, in the evaluation of recurrent or residual mass and in the early restaging of induction chemotherapy in lymphomas, due to the uptake of 2-[fluorine-18] fluoro-2-deoxy-d-glucose (FDG) in malignancies exhibiting increased metabolic activity.[13,14] The effectiveness of FDG-PET to differentiate viable tumor tissue from necrosis or fibrosis has been shown in several studies in the past few years.[2,5,6,16] Literature seems to be consistent in negative predictive value (NPV), however, as regards positive predictive value (PPV), contradictory results are displayed.

In 1994, East Europe's first PET center began to work in Debrecen, Hungary. The residual tumor masses of HL patients have been examined since 1995. The aim of this retrospective study was to assess the value of FDG-PET for prediction of remission or relapse in HL in a rather large cohort of patients after a long follow-up.

MATERIAL AND METHODS

Patients. This study represents a retrospective analysis of 128 patients who had residual masses on CT after completion of their planned treatment. All the patients were treated in the following three institutes: National Institute of Oncology, Budapest; Faculty of Medicine, University of Szeged; Medical and Health Sciences Centre, University of Debrecen. FDG-PET was performed between January 1995 and February 2005. Data collection was finished in January 2008, so

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Table I. Clinical data of 128 Hodgkin lymphoma patients
Abrevations: NLPHL: nodular lymphocyte predominant Hodgkin lymphoma, NS: nodular sclerosis, MC: mixed cellularity, LR: lymphocyte rich, LD: lymphocyte depleted

Age (years)	27 (14-68)
Sex (Male/Female)	63 /65
B symptoms (No/Yes)	64/64
Clinical Stage (Number of patients)	8
I	
II	78
III	29
IV	13
Histology (Number of patients)	
NLPHL	1
NS	98
MC	27
LR	1
LD	1

Table II. Studies of 18-fluoro-deoxy-glucose positron emission tomography (FDG-PET) for restagin of Hodgkin lymphoma (Abrevations: PPV: positive predictive value, NPV: negative predictive value)

Authors	Number of patients	Follow-up (month)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
De Wit et al[6]	33	26	100	78	67	100	85
Ditmann et al[7]	26	6	87	94	87	94	92
Spaepen et al[20]	60	32	50	100	100	91	92
Weihrauch et al[21]	29	28	67	80	60	84	76
Guay et al[9]	48	16	79	97	92	92	92
Friedberg et al[8]	29	24	80	85	50	96	85
Panizo et al [15]	29	28	100	85	75	100	90
Molnar et al	128	75	83	93	74	93	88

time from the end of the examinations to data procession was 35 months, while the mean follow-up of the patients was 75.5 (20-156) months. Clinically complete remission with residual mass was defined by the presence of residual mass on post treatment CT scan, no B symptoms and normal laboratory tests (CRu category). Progression or relapse were defined as histological verification of HL, progression on CT scan, or introduction of a new treatment. The patient characteristics are displayed in Table I.

Patients have been treated and reviewed according to the institutional protocols. Six patients were lost of follow-up. The median time between the end of the treatment and FDG-PET was 3.2 months (range 1.5-5). PET examinations were done with a GE 4096 Plus whole body camera (General Electric). A mean dose (80 mCi-2,96 MBq FDG/body kg) of positron emission FDG 5,4±2,4 mCi (200±89 MBq) was applied. In the case of pharmacon accumulations that could not be explained as focal or physiological variants, decision was made on tumour to background ratio (TBR). If there was no involvement, the appropriate area of the opposite side or the neighbouring soft tissues at about the same depth were taken as background. Based on the results two categories were formed: if the TBR was equal or lower than 3 it was considered as negative, if it was higher than 3 it was positive.[19] The patients with negative result were followed up closely. PET positive patients were treated, if other signs of the active disease were obvious. If no other sign of active disease was found biopsy was done, or thorough observation was chosen according to the clinical situation. We considered the result to false positive when it was caused by non-lymphomal activity. Specificity, sensitivity, PPV, NPV and accuracy have been calculated according to literature.[17] Event free survival (EFS) has been calculated from the date of the PET scan to the date of documented relapse, death, or to the last follow-up visit. EFS and OS rates were estimated by the Kaplan-Meier method. Mantel-Cox c^2 probes were used for analyzing the effect of different parameters (age, stage, histological subtype, the time between the end of treatment and PET and the date of PET examination) on the false results.

RESULTS

The result of FDG-PET examinations was negative by 89 patients (70%) and positive by 39 patients (30%). The number of true-positive (TP), true-negative (TN), false-positive (FP), false-negative (FN) subjects were 29, 83, 10, 6, (22.6, 64.9, 7.8, 4.7 %) respectively, which means that 6.7 % of 89 PET negative cases were FN, while 25.6 % of 39 PET positive cases were FP. Sensitivity of post-treatment FDG-PET was 83 %, specificity 93 %, PPV 74 %, NPV 93 %, and accuracy 88 %. The difference between the EFS of PET positive and negative cases was highly significant (p=0.0000) (Figure 1).

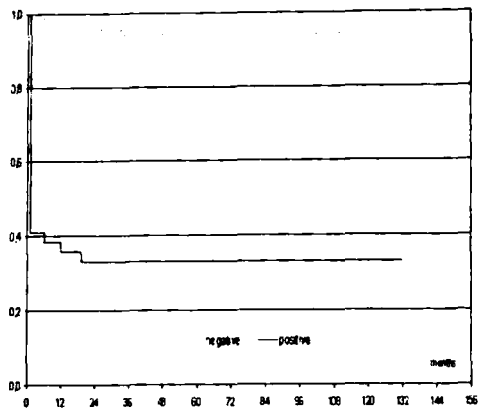

Figure 1. Event free survival according to the PET results in Hodgkin lymphoma patients

In the FN group EFS from PET was 33, 34, 39, 54, 57 and 105 months, respectively. Regarding relapses, in 3 patients they were proved histologically, while in the other 3, with CT scans. Three patients died in this group, all of them because of progressive disease. One achieved a second complete remission (CR), one has nodular lymphocyte predominant HL (NLPHL), he has active disease, but does not need any treatment, and one patient was lost of follow-up with progressive disease, after 54 month. In the TN group the median follow-up time is 67 months (range: 35-151), the results were proved by follow-up data. None of the patients died and 2 of them were lost of follow-up from that group. In 39 cases positive PET results were found, from them, in 10 cases the relapse was not likely, because of the clinical findings, so in these cases thorough observation was chosen without treatment. In these cases during the 89 (49-147) months mean follow-up time no relapse occurred, so the result of PET was false positive. In 24 cases the clinical finding (signs, laboratory results, other radiological methods) corresponded with PET result, so it was accepted, and new treatment was applied. In the five doubtful cases biopsy was done to confirm the relapse. From the 29 TP cases, 9 patients died because of progressive disease, 16 achieved CR with further treatment, and 3 were lost of follow up. Looking for the reason of false results, we examined the association of treatment modalities and PET outcomes, but no significant differences were found. Neither the variety of time from treatment to PET explained these false results. (Data were not collected to separate tables.) We found increased activity of the thymus by three patients, sarcoidosis by two patients and pleuro-pneumonia by one case in the background of false PET positivity. The reason for false positivity cannot be explained from the medical files by the remaining four patients.

DISCUSSION

Before FDG-PET was introduced for the clinical evaluation of patients with HL, tumor activity of residual masses after therapy could only be assessed by invasive procedures or by regularly repeated CT scans. Gallium scintigraphy has been used as a metabolic imaging technique to detect active tumor tissue, but it has several disadvantages.[10] FDG-PET has been reported to be superior to gallium in respect to sensitivity and specificity.[16] In a recent systematic review, Zijlstra[22] and colleagues summarized the results of 15 studies evaluating PET after the first line treatment in lymphoma. Subgroup analysis in 247 HL patients showed a sensitivity and specificity of 84% and 90% for the detection of residual lymphoma; our results are similar to these. The result of this trial clearly indicates that patients with negative FDG-PET results are unlikely to progress or relapse during a long follow-up. However, if FDG-PET was positive after treatment, only about 60 % of patients would relapse. According to our data, in accordance with literature, false positive uptake is a problem in HL patients.[1,4,18] The most frequent causes of false positivity are summarized in Table III.

Table III. The most frequent causes of false positive (non-lymphoma) result of 18 fluoro-deoxy-glucose positron emission tomography (FDG-PET)

sarcoidosis
tuberculosis
histoplasmosis
fungal infections
pyogenic abscess
thymic hyperplasia
hyperplasia of the bone marrow
pneumonitis

Attenuation correction as well as standard uptake value (SUV) were not calculated.[18] The diagnosis of relapse should be confirmed with additional, perhaps invasive diagnostic procedures, or with closer follow-up.[12] If clinical signs do not refer to relapse, it may be considered to repeat the FDG-PET scan within 4-12 weeks. FN results should be eliminated, too, however, relapse was detected among these patients three years or later after the scan. Referring De Wit et al[6], we also think that it is not correct to evaluate false negativity in relapses after three years, as it should be considered to the result of minimal residual disease, which cannot be detected by FDG-PET scan. The GHSG evaluated the NPV of PET scan by advanced stage HL patients in the HD15 survey. They defined the relapse after 12 months as a late event which cannot be predicted by PET. This way, NPV should be adopted only to 12 months progression free survival.[12] However, FDG-PET improves the accuracy of restaging assessment over that of CT alone, so it is accepted as the most valuable tool for HL restaging. The new definition of complete remission is based on its results.[3] Its accuracy can be further improved by the use of PET/CT. It can be concluded that PET examinations help to plan individual, risk-adopted treatment modalities, which improves both

the curability and the long-term quality of life in young people with HL. East-Europe's first PET apparatus was available in Hungary, this is why we were able to present our experiences in a large population during a long follow-up.

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Results of immuno-chemotherapeutic treatment of patients with diffuse large B-cell lymphoma

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Background Treatment with cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) has been considered as the standard therapy for diffuse large B-cell lymphoma (DLBCL) for more than 20 years. CHOP treatment in combination with targeted immunotherapy, rituximab (R-CHOP), resulted in significant improvements in the treatment of this group of patients. In this study, the efficacy of R-CHOP and R-CHOP-like treatments was analysed. The results were compared to the data of historical patients only receiving CHOP treatment or CHOP-like treatment.

Methods Between September 2002 and April 2005, 140 newly diagnosed, untreated DLBCL patients started to receive R-CHOP treatment in a single centre. The results were compared to the data of 130 patients only receiving CHOP treatment in the past.

Results In the patients receiving R-CHOP, the therapeutic outcomes were superior for all parameters. The overall remission rate was 73.6% in the R-CHOP group in comparison with 47.7% in the CHOP group. The 3-year overall survival was 71.7% vs. 50.5% (p<0.0001), the event-free survival was 61.4% vs. 50.5% (p<0.0001) and the progression-free survival was 65.2% vs. 50.5% (P<0.0001). Patient groups were also compared using the International Prognostic Index score.

Conclusion Combining the standard CHOP treatment with rituximab resulted in a significant improvement of the therapeutic outcomes irrespective of the prognostic grouping. The data are comparable with those reported in the international literature.

Key words: diffuse large B-cell lymphoma, immuno-chemotherapy, rituximab

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin's lymphomas (NHL) accounting for approximately 35% of all newly diagnosed cases and for more than 80% of aggressive lymphomas. However, DLBCL is not a homogeneous group of patients.

From the middle of the 1970's until recently, CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) and CHOP-like treatments were considered as the standard therapies for DLBCL.[1,2,6] As a result of receiving these combinations, several patients were definitively cured; however, one third of the patients in complete remission were expected to experience a recurrence of the disease. The 3-year disease-free survival was around 40% and the overall survival was around 50%. Since the early 2000's, the administration of the anti-CD20 monoclonal antibody (rituximab) in combination with the CHOP treatment has resulted in significant improvements in the therapeutic outcomes. Today, this new type of treatment, R-CHOP (rituximab + CHOP) may be considered as the new standard..[4,18,3,7,14,16]

In Hungary, the anti-CD20 monoclonal antibody was authorised for use in the treatment of DLBCL patients in September 2002. In this retrospective study, the authors compared the efficacy of R-CHOP treatment with the outcomes of a historical control group receiving a CHOP regimen and with the data available in the international literature.

MATERIALS AND METHOD

Patients. At the Centre of Malignant Lymphomas of the National Institute of Oncology of Budapest, the authors registered 506 new patients with non-Hodgkin lymphomas between September 2002 and April 2005. 179 (35.3%) of them had diffuse large B-cell lymphoma. Histological classification was performed in accordance with the WHO classification system.[9] Before treatment initiation, routine blood tests, urine tests, chest x-rays, as well as cervical, abdominal and pelvic computer tomography (CT) scans and bone

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marrow biopsies were done. During the restaging of patients with clinical remission after completion of the planned treatment, positron emission tomography (PET or PET/CT) scans were also performed in addition to the above, with the purpose of precisely evaluating the level of remission.[8]

The eligibility criteria included advanced stage (clinical stages III-IV), or large tumour size (>7 cm) and/or symptom B and/or extranodal manifestation in the case of clinical stages I-II. Exclusion criteria included the involvement of the central nervous system and any diseases transforming from indolent lymphomas. Patients who were not eligible for a curative treatment were not included in the study.

140 (78.2%) of the 179 newly diagnosed DLBCL patients were included in the study. The new combination, R-CHOP treatment was administered to these patients. The treatment outcomes of these 140 patients were compared to the results of a group of 130 patients receiving a CHOP or CHOP-like treatment regimen in the past (between April 1996 and August 2002). Table I shows the main clinical parameters detected at the time of the diagnosis.

The average follow-up periods of the groups receiving CHOP and R-CHOP treatment were 66 months and 59 months, respectively. The mean age was equal – 54 years – in the two groups. Gender distribution did not show essential differences. Again, no major differences were found in the proportion of large tumours and patients with elevated LDH levels. However, the proportion of patients in advanced stages and poor general conditions, and thus to be classified to an unfavourable IPI group, was lower in the R-CHOP group.

All patients received curative CHOP or CHOP-like therapy (epirubicin instead of doxorubicin) (hereinafter referred to as 'CHOP') as treatment, repeated in 21-day cycles. In the rituximab group, immunotherapy was administered on the day of receiving the chemotherapy, directly before the CHOP treatment, in a standard dose of 375 mg/m^2 in each cycle. In case of a residual disease upon completing the immuno-chemotherapy, supplementary radiotherapy was applied to the affected region.

In the rituximab group, the therapeutic response was evaluated according to Cheason's criteria and PET scans were also performed to confirm complete remission.[2] The analyses included the expected 3-year overall survival (OS), progression-free survival (PFS) and event-free survival (EFS) of the two patient groups (CHOP and R-CHOP), and the same values according to clinical stage (stage I-II and III-IV), general conditions (PS) and prognostic groups (IPI).

Statistical analyses were performed according to the recommendations of Kaplan and Maier.[11] Prognostic variables were compared between the two groups, using the independent-samples t test for continuous variables and the c^2 test for categoric variables. Data were analized using the BMDP Statistical Software.

RESULTS

Between September 2002 and April 2005, 140 DLBCL patients received standard CHOP treatment combined with rituximab. The treatment outcomes were compared to the results of a group of 130 patients receiving CHOP treatment in the past (between April 1996 and August 2002). The median follow-up period for surviving patients was 66 months for the CHOP arm (range: 2 to 154 months) and 59 months for the R-CHOP arm (range: 3 to 78 months). The outcomes according to treatment type are listed in Table II.

The complete remission (CR) rate was much more favourable in the rituximab arm (73.6% vs. 47.7%), and the relapse rate was only half in the R-CHOP group (13.6% vs. 27.4%). These advantages are also manifested in the overall survival (OS), progression-free survival (PFS) and event-free survival (EFS) of the CHOP and R-CHOP groups. While the 3-year OS was 50.5% in the CHOP arm, it was much higher (71.7%) in the R-CHOP arm ($p<0.0001$). (Figure 1) The 3-year PFS was similarly more favourable in the

Table I. Patient characteristics

Characteristics	CHOP (n=130)	R-CHOP (n=140)	p
Median age, years (range)	54 (17-78)	54 (17-88)	0.4430
Gender, male sex	64 (50 %)	75 (53 %)	0.4758
LDH level > normal	77 (59 %)	89 (63 %)	0.5529
Stage III-IV	87 (64 %)	67 (48 %)	0.0001
Bulky disease > 7 cm	85 (66 %)	90 (64 %)	0.8501
PS ≥ 2	63 (48 %)	46 (32 %)	0.0090
R-IPI (modified by Sehn) score			0.0066
very good	9 (7 %)	21 (15 %)	
good	52 (40 %)	64 (46 %)	
poor	69 (53 %)	52 (37 %)	
unknown	0	3 (2 %)	
Pathology, number of patients			
DLBCL	119	116	
PMBCL	11	24	

Abbreviations: PS: ECOG performance status; LDH: lactate dehydrogenase; IPI: International Prognostic Index; DLBCL: diffuse large B-cell lymphoma; PMBCL. primary mediastinal B-cell lymphoma

Table II. Results according to treatment type

Outcome	CHOP (n=130) (%)	R-CHOP (n=140) (%)	p
All patients [n (%)]			
Alive without lymphoma	32 (24.6)	77 (55.0)	
Alive with lymphoma	4 (3.1)	6 (4.3)	
CR	62 (47.7)	103 (73.6)	
Relapse from CR	17 (27.4)	14 (13.7)	
Death			
Lymphoma related	68 (52.3)	32 (22.8)	
Treatment related	5 (3.8)	6 (4.3)	
Other cause	14 (10.8)	5 (3.6)	
Lost to follow-up	7 (5.4)	14 (10.0)	
3-year OS	130 (50.5)	140 (71.7)	<0.0001
3-year PFS	130 (40.8)	140 (62.2)	<0.0001
3-year EFS	130 (39.0)	140 (61.4)	<0.0001
Survival according to IPI (revised by Sehn)		[n=136]	
Very good risk group			
3-year OS	100.0 % (n= 8)	90.9 % (n=23)	0.3873
3-year EFS	75.0 %	87.0 %	0.1702
Good risk group			
3-year OS	65.5 % (n=53)	76.2 % (n=63)	0.0084
3-year EFS	46.6 %	65.6 %	0.0185
Poor risk group			
3-year OS	33.3 % (n=69)	56.0 % (n=50)	0.0030
3-year EFS	29.0 %	45.3 %	0.0299

Abbreviations: CR: complete remission; OS: overall survival; PFS: progression-free survival; EFS: event-free survival; IPI: International Prognostic Index

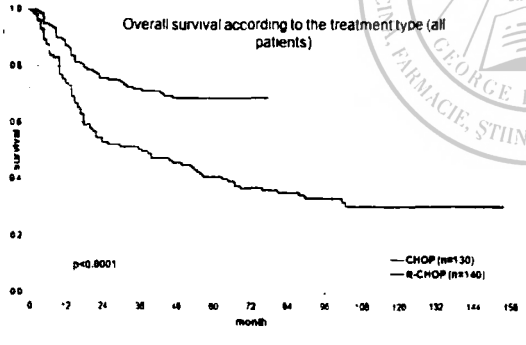

Figure 1.Overall survival according to the treatment type

rituximab group; 40.8% in the CHOP group and 65.2% in the R-CHOP (p<0.0001) (Figure 2). The 3-year EFS was 42.9% in the CHOP group and 61.4% in the rituximab group (p<0.0001).

An assessment of role of the Sehn-revised IPI subgroups revealed that in the groups with good and poor prognoses, rituximab addition to the standard CHOP treatment resulted in strongly significant improvements of the OS, the PFS and the EFS.[15,17] The 3-year OS was 65.6% vs. 76.1% (p=0,0084) and 33.3 % vs. 58.1% (p=0.003) in the group with good prognosis (IPI = 1-2) and in the group with poor prognosis (IPI = 3-5), respectively, to the advantage of the rituximab group (Figure 3 and 4).

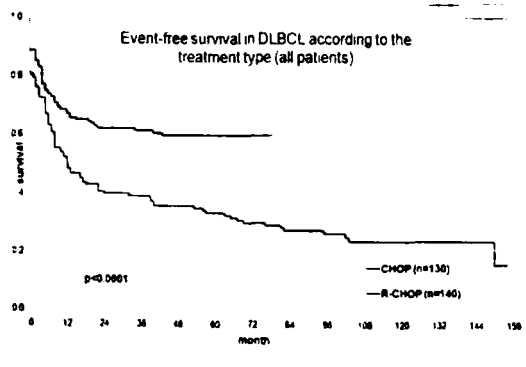

Figure 2. Event-free survival in DLBCL according to the treatment type.

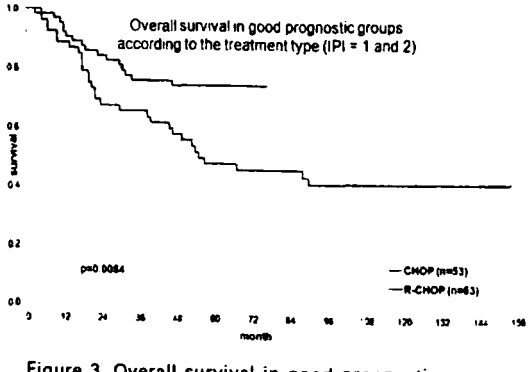

Figure 3 Overall survival in good prognostic groups according to the treatment type (IPI = 1 and 2)

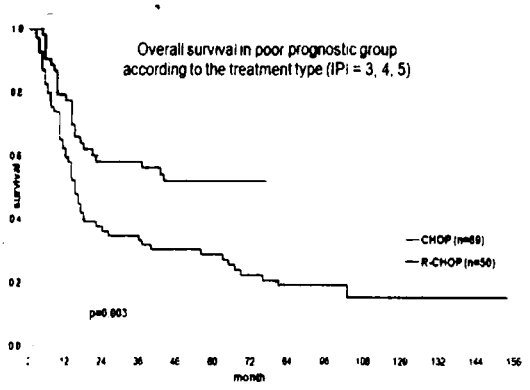

Figure 4. Overall survival in poor prognostic groups according to the treatment type (IPI = 3, 4, 5)

In the group with very good prognosis (IPI = 0), the statistical difference between the two groups in terms of the 3-year survival parameters remained undetectable as a result of the already very high therapeutic effect and low case number (CHOP: 100% vs. R-CHOP: 90.9%; p=0.3873). Similarly favourable changes were detected in the PFS and EFS among the IPI groups, in the good prognosis group (3-year PFS: 48.4% vs. 65.6%; p=0.0531; EFS: 46.6% vs. 65.6%; p=0.0185) and in the poor prognosis group (3-year PFS: 30.92% vs. 53.72%; p=0.0175; EFS: 29.0% vs. 45.3%; p=0.0299). Except for the 'borderline significance' (p=0.0531) between the progression-free survival rates of the groups with good prognosis, the differences are significant. However, the statistical significance may not be proven in the very good prognosis group with low case number (3-year PFS: 75.0% vs. 91.1%; p=0.0812; 3-year EFS: 75.0% vs. 86.9%; p=0.1702). One favourable effect of the new treatment is that the lymphoma-related mortality rate was reduced to less than half in the rituximab arm (52.3% vs. 22,8%) while the treatment-related mortality did not show essential differences (3.8% vs. 4.3%) between the two groups.

DISCUSSION

The therapy of aggressive lymphomas is one of the most successful fields in oncology. The CHOP chemotherapy regimen has been the standard therapy since its development in the middle of 1970's.[12] In a multicentric, prospective study involving a large group of patients published by Fischer et al. in 1993, the 'golden standard' role of CHOP treatment in the first-line treatment of DLBCL was confirmed.[6] High-dose treatments with stem-cell support have been in use since the early 90's but only a small group of selected patients could benefit from that.[13,1] From the early 2000's, new studies were published reporting the results of combining the standard CHOP treatment with rituximab; later, three large, randomised studies and one population-based study also confirmed the efficacy of the new standard, R-CHOP, in DLBCL.[3,7,14,16]

Currently, no data is available regarding the effects of the new standard R-CHOP treatment on the survival of Hungarian patients. The authors found that the outcomes achieved by the administration of R-CHOP were clearly superior to the prior standard CHOP treatment. Upon comparing the various patient groups, the 3-year complete survival (50.5% vs. 71.7%), the progression-free survival (40.8% vs. 62.2%) and the event-free survival (39.0% and 61.4 %) all showed dramatic improvement and high significance ($p<0.0001$) to the advantage of the R-CHOP arm. Among the patients receiving R-CHOP, an additional 26% achieved complete remission (47.7% vs. 73.6%). The significantly less recurrence from complete remission also contributed to the improvement of the therapeutic outcomes. In the rituximab arm, the relapse rate was reduced by about half (27.4% vs. 13.7%). The difference between the two groups is also significant in terms of the lymphoma-related mortality (52.3% vs. 22.8%). Subgroup analyses revealed significantly increased efficacy in the rituximab group.

Our results are comparable with the international results of the GELA and ECOG studies involving older patients and those of the MInT study, which processed the treatment data of young patients with favourable prognoses are completely comparable and almost equal to the results of the present study.[3,5,7,14,16]

CONCLUSIONS

The administration of rituximab in combination with the standard CHOP treatment resulted in considerable, statistically significant improvements in the therapeutic outcomes of DLBCL patients. This favourable effect was detectable in the overall group of patients receiving R-CHOP, as well as in almost all analysed subgroups. The study confirmed the favourable joint effect of immune-chemotherapy on DLBCL. The results are in compliance with those reported in the international literature. The already very good treatment results could be further improved by the use of interim PET/CT scans functioning as early indicators of the therapeutic efficacy and the dose-dense treatment of patients with poor prognosis.

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BRCA1 testing in breast cancer following Hodgkin's lymphoma

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The risk of radiation-induced breast cancer in women treated for Hodgkin's lymphoma (HL) is well known, however the importance of genetic factors in the aetiology of secondary breast cancer is not yet established. We have studied whether there is an association between the development of breast cancer after radiation treatment for HL and germ line mutation in the BRCA1 breast cancer-susceptibility gene. We performed mutation analyses of the BRCA1 gene in two female patients with secondary breast cancer after HL. These patients had a positive family history of breast cancer and they received chest irradiation for HL. We used PCR-SSCP methods for screening the BRCA1 mutation in the two HL patients and in their family members. We identified germ line BRCA1 mutation in the first HL patient. She was irradiated at the age of 19 and the latency to breast cancer was only 5 years – the mean time to breast cancer following radiation is approximately 15 years in the literature. The second HL patient had not mutation in the BRCA1 tumour suppressor gene but one of her family member suffering with non-Hodgkin's lymphoma carried germ line BRCA1 mutation. This study suggests that several factors contributing to second cancer risk include the carcinogenic effect of ionizing radiation in combination with possible host susceptibility. Radiation treatment of HL and mutation in the BRCA1 gene together is associated with a higher risk of breast cancer. BRCA1 mutation testing at the diagnosis of HL for female patients with familial aggregation of breast cancer is useful to identify the high risk group. Future consideration for treatment and guidelines for follow-up of these patients may reduce the occurrence of secondary breast cancer.

***Key words**: Hodgkin' lymphoma, radiation treatment, secondary breast cancer, BRCA1 gene*

During the past three decades, advances in the diagnostic procedures and the use of chemo- and radiotherapy, the survival of patients with Hodgkin's lymphoma has significantly increased, with overall survival rates of eighty percent to eighty-five percent at 10 and 20 years. The treatment of Hodgkin's lymphoma has been one of the great success stories in the field of oncology but the occurrence of late complications has now been recognized as a major problem. Second malignancies are now the leading cause of death in long-term survivors of HL, with secondary breast cancer representing the most frequent solid tumour among women.[6,8] The risk of radiation-induced breast cancer in women treated for Hodgkin's lymphoma is well known, however the importance of genetic factors in the aetiology of secondary breast cancer is not established yet.[3,5]

MATERIALS AND METHODS

We have studied whether there is a link between the development of breast cancer after radiation treatment for HL and germ line mutation in the BRCA1 breast cancer-susceptibility gene. We have performed mutation analyses of the BRCA1 gene in two female patients with secondary breast cancer after HL. These patients had positive family histories of breast cancer and they received chest irradiation for HL. We used PCR-SSCP methods for screening the BRCA1 mutation in the two HL patients and in their family members. Mutation analysis of each patient was done after genetic counselling, with the approval of the Institutional Ethical Board. For mutation screening blood samples were obtained, and genomic DNA was isolated using a standard phenol-chloroform method. The entire coding region and splice sites of the BRCA1 gene were amplified by PCR and subjected to conformation-sensitive gel electrophoreses (HDA; heteroduplex analysis as well as SSCP; single-strand conformation polymorphism analysis) and denaturing high-performance liquid chromatography (DHPLC) asprescreening. All samples showing altered migration patterns on the gels or retention times different from that of a negative control were sequenced bidirectional using Big Dye Terminator version 1.1 Cycle Sequencing Kit on an ABI 310 Genetic Analyzer (Applied Biosytems). All varians were ascertained by a replicate experiment.

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RESULTS AND DISCUSSIONS

We performed mutation analyses of the BRCA1 gene in two female patients with secondary breast cancer after HL. These patients had a positive family history of breast cancer and they received chest irradiation for HL.

The first patient (L.Zs.) was 19 years at diagnosis of HL; she received multiagent chemotherapy and involved field's radiotherapy. The latency to secondary breast cancer was quite short, only 5 years.

The clinical characteristics of the first patient are presented in Table I.

We identified germ line BRCA1 mutation in first HL patient. The electrophoretogram shows the migration patterns of normal samples and the mutation carrier (marked by a star) (Figure1).

The family tree of the first patient is shown in Figure 2.

The second patient (F.P.) was middle age at the time of diagnosis of HL, she received chemo- and radiotherapy as well. The interval until the development of second malignancy was longer, more than 10 years. The clinical characteristic of the second patient are given in Table II.

The second HL patient had not mutation in the BRCA1 tumour suppressor gene but one of her family member suffering with non-Hodgkin's lymphoma carried germ line BRCA1 mutation. The family tree of the second patient is shown in Figure 3.

Table I. The clinical characteristics of the first patient (L.Zs.)(ABVD – doxorubicin,bleomycin,vinblastin,dacarbazine)

Age at HL 19 years
Hodgkin's lymphoma - nodular sclerosis Stage II.B.
Treatment : chemotherapy ABVD (6x)
radiotherapy chest, neck 30 Gy
Complete remission from 2000.
Secondary tumour after 5 years
Ductal infiltrating carcinoma gr.III. pT2pNo(b)Mx St. II.
Oestrogen receptor negative
Treatment : mastectomy and chemotherapy

Figure 1. SSCP, DHPLC and direct sequencing = L.Zs BRCA1 mutation

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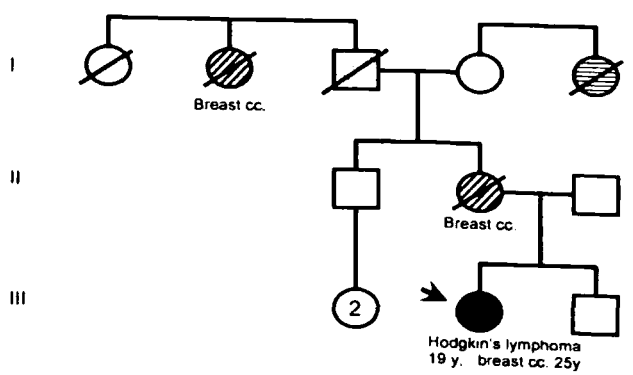

Figure 2. The family tree of the first patient (L.Zs.)(The black dot in the tree shows the patient)

Table II. The clinical characteristics of the second patient (F.P.) (ABV – doxorubicin,bleomycin,vinblastin)

Age at HL 43 years
Hodgkin's disease -mixed cellularity (1988) Stage I.A.
Treatment: radiotherapy chest 40 Gy
neck 42 Gy
chemotherapyABV (6x)
Complete remission from 1993
Secondary tumor after 10 years
Ductal infiltrating carcinoma pT1pN1(b)Mo St. II.
Treatment: quadrantectomy + axill. blockdissectio
radiotherapy 50 Gy + Tamoxifen

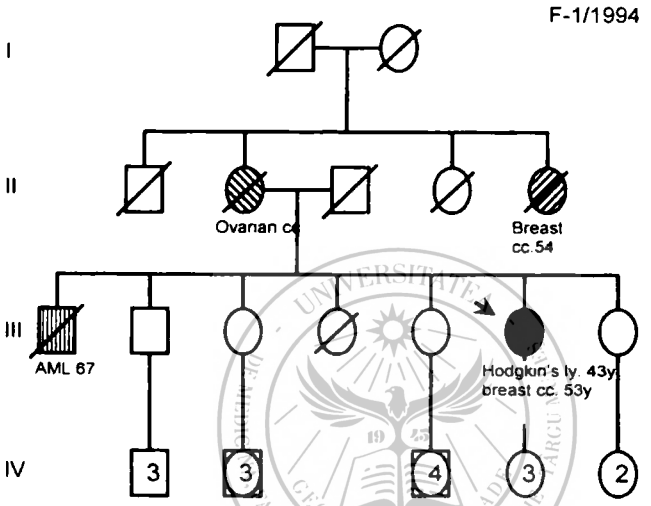

Figure 3. The family tree of the second patient (F.P.) (The black dot in the tree shows the patient)

As to the evidence of large centres the risk of secondary solid tumour is the lowest in patients treated only with chemotherapy, and it is high after radiotherapy and similarly high in those receiving both chemo- and radiotherapy.[2] Our tested female patients received both chemo- and radiotherapy. Secondary solid tumours can frequently be observed in the regions irradiated. e.g. chest irradiation and mamma carcinoma or cervical irradiation and thyroid carcinoma.[8] In our two patients, secondary breast cancer developed in the region irradiated earlier, so we have seen relationship between the site of solid tumour and the irradiated region. The general tendency in radiotherapy, to minimize radiation dose to surrounding normal tissues and recently, also to decrease the tumour dose.[8] Most publications considered the age above 40 years significantly risk-increasing while in case of some secondary solid tumours irradiation performed at young age - associated with higher risk. e.g. chest irradiation of women below 20 years of age and breast cancer.[1,4,9] The first patient (L.Zs.) irradiated at very young age – she had positive family history of early breast cancer and she carried BRCA1 mutation – so these factors associated with very high risk of secondary breast cancer. The second patient also had positive family history of early breast cancer but she had received irradiation at age above than 40 and she had no mutation BRCA1 tumour suppressor gene - so she an increased risk of breast malignancy but not as extremely high as the first patient's. Several studies recommends breast cancer screening for woman treated for HL using radiotherapy - a baseline mammogram 5 to 8 years following initial treatment, repeated every 3 years till age of 40, and than annually.[1,7]

CONCLUSIONS

This study suggests that several factors contributing to second cancer risk include the carcinogenic effect of irradiation in combination with possible host susceptibility.

Radiation treatment of HL and mutation in the BRCA1 gene together is associated with a higher risk of breast cancer. BRCA1 mutation testing at the diagnosis of HL for female patients with familial aggregation of breast cancer is useful to identify the high risk group. Future consideration for treatment and guidelines for follow-up of these patients may reduce the occurrence of secondary breast cancer. The

continuous follow-up of patients in remission and careful evaluation of the clinical symptoms are necessary for the early diagnosis and therapy of potential secondary tumours.

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Human Immunodeficiency Virus Infection and surgery

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Introduction. HIV infection has a chronic evolution, it is estimated that between 20%-25% of HIV infected patients require surgery sometimes during their illness. The objective of the study was to evaluate the morbidity of surgical intervention and cross infection risks to surgical teams. Material and method. A retrospective study of records of 39 HIV-infected individuals between 17 and 43 years old, in stages B2-C3, who underwent surgical procedures in last 2 years. The type of surgical procedures, postoperative evolution, corroborated with the level of immunodeficiency, the occupational exposure to blood has been evaluated. Results. 42 interventions have been made, with diagnostic, therapeutic, prophylactic and esthetical reason 12, 13, 15 and 2 respectively. No significant complications have been registered, nor deaths, although the majority of patients had advanced immunodeficyency, detectable viral charge, without ARV therapy. Three occupational exposures have been signaled. Everyone benefited of prophylactic treatment and remained seronegative. Conclusions. HIV infected patients even in advanced stages can benefit from the surgical treatment with good results. HIV infection isn't an independent surgical risk factor. Post exposure profylaxis is efficient.

***Key words**: HIV, surgery, outcome, risk*

Infection with HIV (HIV) has become a global public health problem. In 2008 more than 40 million people worldwide were infected with HIV. The modern antiviral therapies, HAART, have lead to a more-chronic evolution of this disease, with a significantly improved life expectancy and quality of life. It is estimated that between 20% and 25% of HIV/AIDS patients require surgery sometimes during their illness, for problems both related and unrelated to HIV infection.[1,5] The objective of the study was to evaluate the type of surgical procedure, to determine if HIV infection is an independent risk factor for complications of surgery, to evaluate the cross infection risks to surgical teams, to report our experience with this patient population.

MATERIAL AND METHOD

We conducted a retrospective study of 39 HIV-infected individuals followed up at Infectious Diseases Clinic I. Tg.Mures, who underwent surgery procedures from 2007 trough 2008. We used the records of these patients with respect of the confidentiality of personal health information. The surgical affections character, intraoperatory and postoperative evolution, complications have been corroborated with the level of immunodeficiency, viral load, antiretroviral treatment (ARV). The Lymphocyte CD4 (CD4) cell count less than 100/mmc and viral load (V.L) greater than 30000 copies/mmc we consider risk factor. We evaluated the surgical risk using the NNISS (National Nosocomial Infections Survey System) score, and the risk of occupational transmission of HIV to the surgical team.

RESULTS AND DISCUSSIONS

A total of 42 surgical interventions: 15 prophylactic, 13 therapeutic, 12 diagnostic, and 2 esthetical procedures have been performed. The prophylactic interventions was made to prevent maternal-to-child transmission (MCT), all of the procedures have been programmed ahead (Table I).

At this category of patients we don't observe any complications. All of them received antibiotic prophylaxis.[3,7] These interventions reduce successfully the MCT.

Surgical interventions for diagnostic reasons were made to 12 patients (Table II).

*One patient had a wound infection with methicillin resistant Staphylococcus aureus, he needed readmission and antibiotic therapy, the second one had hygroma resolved with local therapy by needle aspiration.

Histological exam's evidenced in 8 cases ganglionar tuberculosis. The open lymph node biopsy is indicated in suspicion of tuberculosis and lymphoma. All the patients received antibiotic prophylaxis.

The surgical interventions for therapeutic reasons were performed at 13 patients (Table III).

*Five patients had appendectomy for acute appendicitis, one thoracotomy was performed for tuberculoma, one thoracoplasty was performed due to pneumococcal empyema, and one exploratory

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Table I. Demographic characteristics, risk factors of interventions to prevent MTC

Type	Nr	Average age/year	Stage			CD4 <100/ μL	V.L> 30.000/ μL	ARV	NNISS
			B2	C2	C3				
Caesarean section	10	18,6	3	2	5	4	6	4	0
Therapeutic abortion	5	19,2	1	1	3	0	1	4	0

Table II. Demographic characteristics, risk factors, and outcomes of diagnostic interventions

Type	Nr	Average age/year	Stage			CD4 <100 / μL	V.L> 30.000/μL	ART	NNISS	Complications
			B3	C2	C3					
lymph node biopsy	11	19,25	2	1	8	4	4	4	0	2*
Skin biopsy	1	19	1	1	-	0	1	1	0	-

Table III. Demographic characteristics, risk factors, and outcomes of therapeutic interventions

Type	Nr	Averag e /year	Stage			CD4 <100/μL	V.L> 30.000/ μL	ART	NNISS			Compli-cations
			B3	C2	C3				1	2	3	
Thoraco-abdominal*	9	18,5		3	6	4	3	6	4	1	3	3**
Anorectal surgery	1	43			1	1	1	-		1		-
Nephrotomy	1	18		1		-	1	1		1		-
Skin-abscess	1	19	1			-	1	1		0		-
Hip replacement	1	18			1	-	-	1		1		-

laparotomy was made due to intra-abdominal tumour, associated with partial mastectomy. Cholecystectomy was performed for acute cholecystopancreatitis.

**One patient had intraoperatory bleeding which required immediate intervention with haemostatics. Two patients had delayed wound healing; the threads were removed after 14 days. All the patients were supervised after surgical intervention in intensive care unit; they received broad spectrum antibiotics according to their Carmeli score.

Surgical interventions for esthetical reasons where performed at two patients. One 16 years old in C3 stage for ectropion correction at the right eye, the other 20 year old patient in stage C3 for facial sebaceous cyst. Both of them had undetectable viral load and LTCD4 > 100/mmc. The evolution was favourable.

Occupational exposure to blood from the surgical team and staff were signaled in three cases. Two of them, a plastic surgeon and a gynaecological internist had skin exposure, but the surgical assistant had deep large bore needle stick injury.

After assessing the exposure code and status of source all of them have beneficiated of post exposure prophylaxis (PEP) with antiretroviral drugs for 28 days. They were under observation for 6 months; all of them have remained seronegative. The risk of HIV transmission with sharps injury from infected source is estimated to 0, 3%.[6]

The most frequent surgical procedures were for the prophylaxis of MCT and less frequent those made for esthetical reason. Only one patient had intraoperatory complication which needs quick intervention. Postoperative evolution was good, complications have been observed just in 4 cases.[2,4] The antiretroviral treatment (ARVT) didn't influenced the evolution, 4 patients from those with complication had ARVT. The CD4 cell count was less than 100/ mL in 13 patients and 2 of them had complications, viral load greater than 30000 copies/ mL in 18 patients and 3 of them had complications. We performed Fisher test and we not found statistically significant differences in outcome (p=0, 63). The evaluation of surgical risk using the NNISS (National Nosocomial Infections Survey System) is useful, the risk score>2 is directly correlated with complications. There is a real risk of getting HIV infection to the surgical staff. In our study the PEP was effective. We think that our results were influenced by the low number of studied population, and by the variability of surgical procedures. For further information we need case-control studies.

CONCLUSIONS

The HIV infected patients (even in advanced stage of illness) can benefit from surgical treatment with good results. HIV infection isn't an independent surgical risk factor. The prophylactic treatment of occupational exposure is essential.

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Considerations about bacterial infections on HIV infected adults

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Bacterial infections in HIV infected hosts are characterised by severity and reccurence. Methods: retrospective study (January – December 2007); we have annalysed two groups of patients (Px): A – 141 HIV-infected Px vs B – 4125 HIV-negative Px admitted into Infectious Diseases Department, Hospital 3 Craiova; our aim is to comparatively annalyse incidence, etiological aspects and evolutions of bacterial infections between those two groups. Results: bacterial infections has been identified in 21.98% (group A) vs 9.16% of cases (group B), p<0.001; RR=2,72. Mycobacterium tuberculosis (23.68%) dominated the etiology of bacterial infections in group A, followed by Staphyloccocus aureus (21.05%) and Streptococcus pneumoniae (18.42%), while in group B Escherichia coli (36.77%) and Streptococcus pneumoniae (16.13%) prevails; between the two groups sepsis criteria has been encountered in 8.5% of cases (group A) vs 2.5% (group B), p<0.05; RR=3,02; 14.89% of Px deceased in group A vs 4.71% in group B. Conclusion: HIV infection poses a high risk for severe bacterial infections.

Key words: HIV infection, bacterial infection

The correlation between the appearance and severity of the infections to the immunocompromised host is a harsh reality, and at the present, when, by several causes, this category of patients is continuously rising.[5] The HIV/AIDS infections still have an important place in this field, but with all the progresses related to the antiretroviral therapy, the opportunistic infections as well as those with mutual germs are still present, with severe or relapsed evolutionary potential.[1,5,11]

The bacterias occupy the first place in human's infections and don't lose ground in this way, not even to the seropositive patients.

This way it can be said that the bacteriological infections (BI) also present a contemporaneous issue, by the important changes occurred during the last decade, related to the resistance to antibiotics, the increase of life duration, the diversification of medical services, aspects which are frequently found with seropositive patients and consequently lead to an important percentage of morbidity and mortality.[5]

The prompt and correct diagnostic, by establishing the etiology, is essential to the favorable evolution of the bacteriological infections to adult patients immunocompromised by HIV.

Objectives: The analysis of the incidence, the spectrum and the evolutionary aspects of the bacterial infections to the adult patients immunocompromised by HIV.

MATERIAL AND METHOD

Comparative, retrospective, cohort type study, during January-December 2007, enhancing 2 groups of patients: group A, with 141 adults infected with HIV and group B enhancing 4125 adults non HIV, who were hospitalized in the Clinical Hospital of Infectious Diseases from Craiova during the study period.

In each of the two groups I analyzed the bacterial infections from the point of view of the appearance frequency, of the spectrum and evolutionary aspects.

We surveyed only those bacterial infectious diseases which etiology has been bacteriologically proved.

In group A we analyzed the demographical data and the classification on clinical and immunological stages of the disease.

The statistic analysis of the data has been made using the application Microsoft Excel, the significance of the correlations has been estimated by calculating the statistic significance threshold, using the Fisher test and calculating the relative risk.

RESULTS

In group A, the median of the age is 22,38 years, with limits comprised between 17 and 65 years, and the distribution on genders relatively homogeneous, 51,77% males versus 48,23% females.

The distribution of the seropositive patients enhanced in the group to be studied depending on the clinical and immunological classification was as it follows: 74,4% in C3 stage, 6,38% in C2 stage, 0,71% in C1 stage, 3,54% in B2 stage and 14,89% in B3 stage.

21,98% of the patients from the total of those enhanced in group A were diagnosed with bacterial infectious diseases, with an approximatively equal distribution on genders (48,38% males versus 51,62% females), the majority being in C3 clinical and immunological stage (77,41%) (Table I).

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Table I. Morbidity by bacterial infections in group A vs. group B

	Group A	Group B
IB "+"	31 (21,98%)	378 (9,16%)
IB "–"	110 (78,02%)	3747 (90,84%)

The etiology of the bacterial infections has been dominated by the triad Mycobacterium tuberculosis (23,68%), Stafilococcus aureus (21,05%), Streptococcus pneumoniae (18,42%) (Diagram 1).

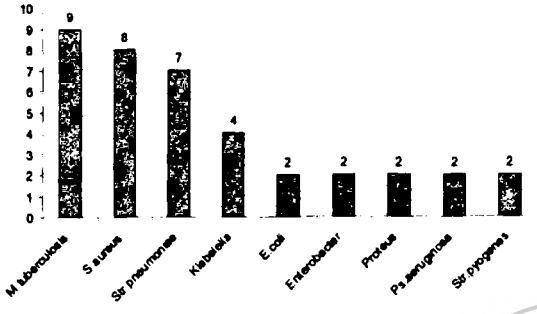

Diagram 1. Group A - Etiological aspects of bacterial infections

The bacterial infectious diseases in group A were represented by: respiratory (64,51%), cutaneous (25,81%), urinary (6,45%) and bone (3,23%) infections.

9,16% from the patients from group B were found with bacterial infections, the etiology being dominated by: Escherichia coli (33,77%), followed by Streptococcus pneumoniae (16,13%), Enterobacter (11,11%) and so on (diagram 2).

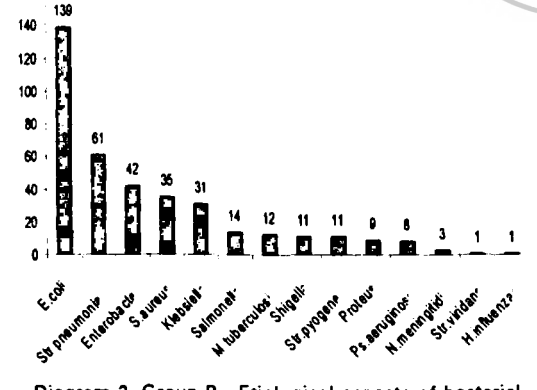

Diagram 2. Group B - Etiological aspects of bacterial infections

The appearance risk of the bacterial infections was higher for the patients infected with HIV versus non-HIV (RR = 2,72, $p < 0,001$).

Recurrences of the bacterial infections were found only in 2 patients from group A, both in C3 clinical and immunological stage, who presented pneumonias with Streptococcus pneumoniae, respectively Klebsiella pneumoniae.

7 of the patients of group A presented 2 acute bacterial infectious sequences, during a year, determined by different diseases, of different etiology.

We have analyzed the criteria of comparative sepsis, between the two groups, tracking that 8,5% of the bacterial sepsis cases in group A versus 2,5% in group B (RR = 3,02, $p < 0,005$).

36,87% of the patients from group A met the sepsis criteria, 8,5% of which were sepsis cases with a mentioned bacterial etiology, Streptococcus pneumoniae being the most frequently involved etiological agent, followed by Mycobacterium tuberculosis and Staphylococcus aureus at the same (Diagrams 3 and 4).

5,18% of the patients from the group B met the sepsis criteria, 2,5 of which were cases of sepsis with mentioned bacteriological etiology, the negative gram bacterias representing their majority etiology (Diagrams 5 and 6).

In group A, 12 patients represented new diagnosed cases with HIV infection (8,51% of the total of the

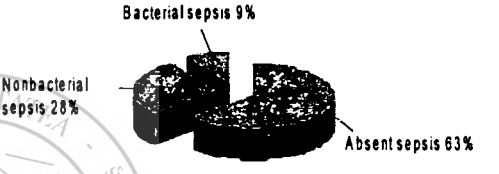

Diagram 3. Group A - Incidence of sepsis

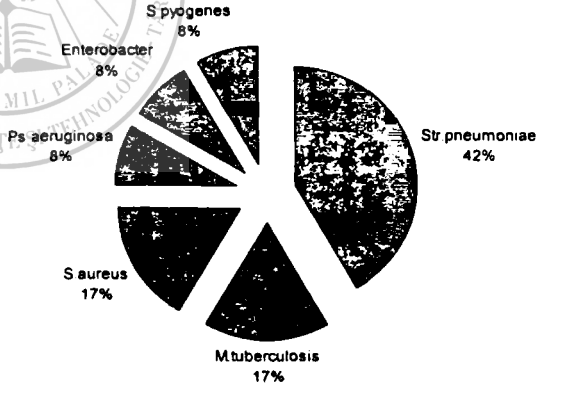

Diagram 4. Group A - Etiological aspects of bacterial sepsis

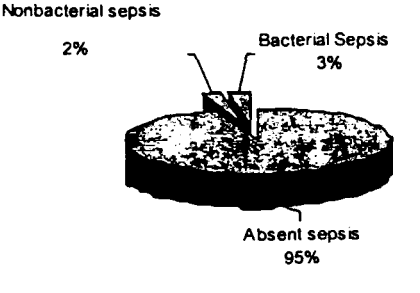

Diagram 5. Group B - Incidence of sepsis

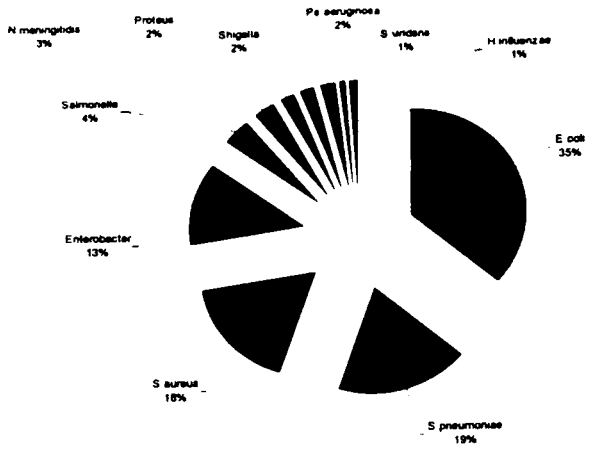

Diagram 6. Group B - Etiological aspects of bacterial sepsis

hospitalizations), the diseases present on diagnostics being probably bacterial pneumonias in 5 cases, the pulmonary tuberculosis in 7 cases (5 cases having a bacteriological proved etiology).

14,98 of the patients from group A died, among which 4,25% presented proven bacterial infections, and 2 of the patients had pulmonary tuberculosis bacterial proven, this representing the disease which lead to the failure (1,41%).

DISCUSSION

The bacterial infections represent an important health issue to the patients HIV infected[1,5], although the number of diagnosed cases is relatively low, and probably under-estimated, probably due to the fact that many of the patients take benefit of antibiotics prophylaxis.

The bacterial pneumonia is an important morbidity and mortality cause to the adults HIV infected.[1,3,7]

Streptococcus pneumoniae was the most frequent cause of community acute pneumonia to both groups of patients (18,42% in group A versus 16,13% in group B), similar to the data of specialty literature.[1,11]

There are controversies regarding the etiology of bacterial pneumonia to the patients HIV infected, the majority of the researchers saying that Streptococcus pneumoniae is the most frequent cause of them[1,11], others supporting the major role of Pseudomonas aeruginosa[2]. In our study, Streptococcus pneumoniae is the majority etiology of the bacterial pneumonias to the seropositive patients, not being diagnosed pneumonias with Pseudomonas aeruginosa.

Pneumococcal pneumonia and pulmonary tuberculosis prove to be the most frequent bacterial infectious diseases to the seropositive patients in accordance with other research studies[7,8,10] but also the most frequent sepsis causes along with the staphylococcus cutaneous infections.

The second cause of the bacterial infectious diseases to the seropositive patients was represented by the cutaneous infections most of them being determined by Staphylococcus aureus, a mutual cause of the dermatological displays associated to HIV, of infectious cause, a similar aspect to the data actually existing in the specialty literature.[1,6]

CONCLUSIONS

1. The patients HIV infected present a high risk of appearance and severe evolution of the bacterial infections.

2. Within the study, the etiology of bacterial infections to the seropositive patients was dominated by Mycobacterium tuberculosis, Staphylococcus aureus and Streptococcus pneumoniae.

3. The respiratory infectious diseases of bacterial etiology represent causes of morbidity – mortality of the seropositive patients.

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Assessment of measles surveillance system for counties in Romania 7 central region

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Measles persists existing in Romania in spite of the fact that the children vaccination against it was introduced by the National System of Immunization 30 years ago. The objectives of this research consist both of monitoring and describing measles cases reported by the National System of Immunization in 2005, 2006 and 2007 and evaluating the actual surveillance system and intervention measures needed to eradicate measles by 2010.

The required information were provided by institutions of a epidemiologic section in six counties. The cases were declared in accordance with "confirmed case" definition.

For the first years included in study, 249 cases were reported, other 535 cases, with an incidence varying from 0.2 $\%_{000}$ (Alba), to 98.4 $\%_{000}$ (Harghita 2005), or 75.3 $\%_{000}$ (Brasov 2006). For the year 2007 a decreasing tendency of cases was noticed. The measles epidemic proportion was firstly caused by discontinuity in providing measles vaccines, a low vaccination coverage in the previous years of 2005, the high percentage of unvaccinated children coming from hard to access communities as well as the tendency of disregarding isolation precautions.

***Key words:** Measles, epidemy, surveillance system*

In 2002 the Regional Committee of WHO for the European Region revised target in order to eliminate indigenous measles which for the European states prolongs to 2010, by using a immunization combination strategy of routine and addition as well as monitoring progress to reach this goal by improving the surveillance system.[3,4]

The surveillance system provides information concerning early detection in order to get quick respond for health events, as well as the epidemic outbreak of the disease. The system provides valuable information to establish priorities, including and allocating budgets for the prevention programs and for the evaluation of the control measures.

For measles there are three major objectives:

-monitoring cases and disease focus

-monitoring events on vaccination including the vaccination coverage and supervising the undesirable post vaccination side effects.

-evaluating the mighty mass of population.[6]

The research objectives:

-Monitoring the measles cases reported by the national system of surveillance between 2005 -2007.

-Describing the epidemic from 2005-2006 and testing the information providing by all the county Health Authorities towards the Center of Preventing and Controlling Catching Disease.

-Appreciating the actual surveillance system and evaluating the intervention measures needed to eradicate measles by 2010.

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MATERIAL AND METHOD

In order to accomplish this objectives, for measles, the data sources were indicated in the case papers provided by the Health Authorities of the six counties included in the research (Alba, Brasov, Covasna, Harghita, Mures and Sibiu), by the Romanian Statistic Yearbook in 2007 and by The Bucharest Institute of Health.[5,6]

The case papers represented sources of information in appreciating the patients' vaccination status, vaccination coverage, and allowed us to correlate these with disease incidence.

The cases were declared by "confirmed case" definition, confirmation made by the present of markers in the first stages of the illness, a IgM antibodies and the epidemiological connection with a confirmed case.

The cases were separated by age: under 1 year, 1-4 years, 5-9 years, 10-14 years, 15-19 years and older than 20 years.

Date sources on population: The Statistic County Departments, The National Statistic Institute and Romanian Statistic Yearbook, for 2007.

RESULTS

Year 2005. From the very beginning of the measles epidemy in Romania, between January 2005 and 31 December 2005 for The Central Region of the country, including those six counties, they made a serologic, clinic and epidemiologic confirmation of 249 cases, most of them in Harghita county 245 (98.4 %), two cases in Mures county and one case in Alba county and Brasov county (table I).

Table I. Number of confirmed measles cases by age groups to 01.01.2006

county / age	< 1 year	1-4 years	5-9 years	10-14 years	15-19 years	> 20 years	Total
AB	1	0	0	0	0	0	1
BV	1	0	0	0	0	0	1
HR	43	126	54	5	7	10	245
MS	1	0	0	0	0	1	2
Total	46	126	54	5	7	11	249

The distribution of these 249 confirmed measles according to the rash month is illustrated by figure 1, the first cases appearing in June, most of following cases reported in the last quarter of the year.

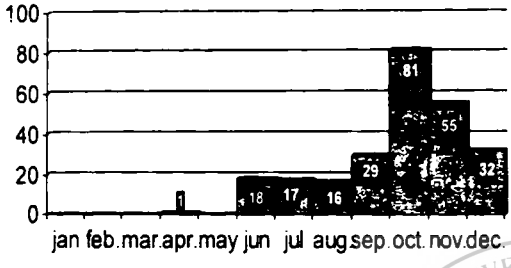

Figure 1. Distribution of measles cases by the rash month during January-December 2005

By analyzing the centralized the information for The Bucharest Institute of Health, rate of incidence by age allowed us to order these groups by affection degree. Therefore, the first place is representing by the under 1 age (R=240.2 $\%_{000}$) then 1-4 year age group (R=165.2 $\%_{000}$) and 5-9 year age group (R=57.1 $\%_{000}$). The cases incidence for the counties was 0.2 $\%_{000}$ for Alba; 0.2 $\%_{000}$ for Brasov; 0.3 $\%_{000}$ for Mures and 75.0 $\%_{000}$ for Harghita. For Harghita county the incidence was higher than the one for the country (22.6 $\%_{000}$), most of cases being reported for the 1-4 year age group (126 cases). The results obtained by correlating relevance of measles confirmed cases by age groups and vaccination states are indicated in figure 2. All the under one year old children received no vaccine dose, and for 1-9 group of age, 149 children were not vaccinated (82.7 %), 30 of them got a vaccine dose (16.6 %), and only a single case was registered with a double dose.

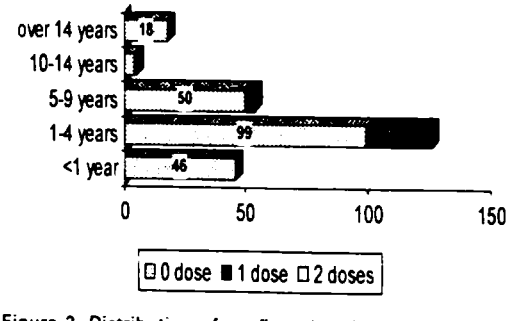

Figure 2. Distribution of confirmed and reported measles cases by vaccination states and age groups, 2005

Year 2006. During that year for the six counties they made a serologic, clinic and epidemiologic conformation of 535 cases, most of them in Brasov county, (75.3 % of all). They also reported many cases Sibiu (80) and Mures (35) county (Table II).

Table II. Number of confirmed measles cases by age groups to 01.01.2007

county / age	< 1 year	1-4 years	5-9 years	10-14 years	15-19 years	>20 years	Total
BV	118	140	78	17	17	33	403
CV	2	7	1	0	0	0	10
HR	1	1	1	0	1	3	7
MS	7	14	5	2	1	6	35
SB	28	29	5	4	8	6	80
Total	156	191	90	23	27	48	535

In the first semester of the year they noticed a large number of diseased, continued the ascending tendency of 2005, and then a decreasing tendency to 61 cases, confirmed for the last three months of the year (figure 3).

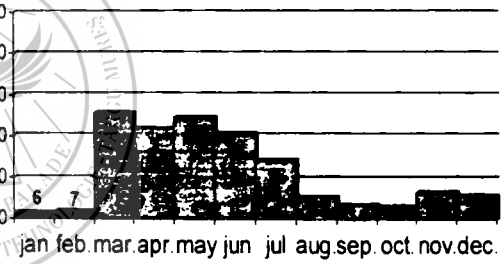

Figure 3. Number of reported measles cases by the rash month during January-December 2006

The same as the previous year, ordering by age is presented as follows: the first place is occupied by the under one year age group (693.1%$_{000}$), then 1-4 year age group (211.4 $\%_{000}$), 5-9 year age group (81.3 $\%_{000}$), 10-14 year age group (20.3 $\%_{000}$) and 15-19 year age group (17.5 $\%_{000}$).The highest rates of incidence were in Brasov (68 $\%_{000}$) and Sibiu (19 $\%_{000}$) counties, many cases were there for the 1-4 year age group.

The correlation of relevance for the reported and confirmed measles cases by age with vaccination states indicates:

-for the under one year age group, 127 cases were unvaccinated, many cases under seven months age, so they could not be vaccinated, only 29 children got a vaccine dose.

-for the 1-9 year age group, 122 children (43.4 %) were identified unvaccinated, 144 (51.2 %) out of them were vaccinated with single dose and 15 cases only were double dose vaccinated (figure 4).

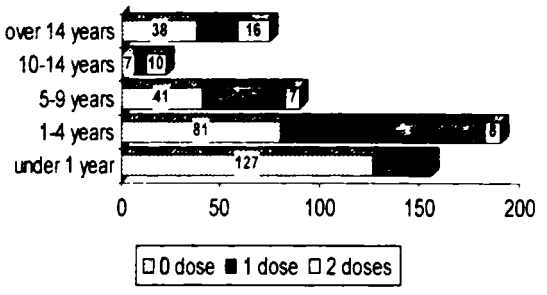

Figure 4. Distribution of confirmed and reported measles cases by vaccination states and age groups, 2006

For 2007 they made a serologic, clinic and epidemiologic confirmation of much less cases in comparison with the previous years (85), most of them in Brasov (72 cases) and Covasna (13 cases) counties and in the first quarter of the year.

The vaccination covering for 2005-2007 oscillated from 77.4 % (2005-Harghita) to 98.6 % (2005-Mures), the best average value registered for Mures county (98.2 %) (Figure 5).

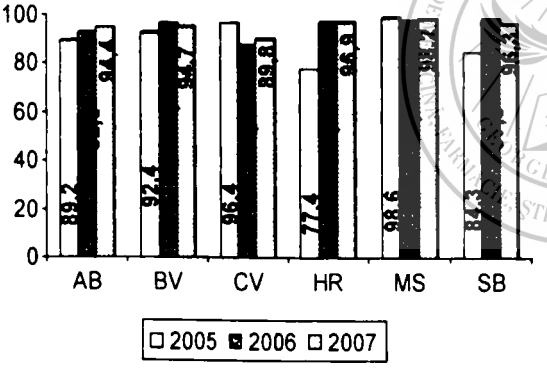

Figure 5. Vaccination coverage for measles during 2005-2007

DISCUSSION

The reported data reveal that the incidence varies from less than 0.2 per 100000 persons (Alba), to 98.4 $\%_{000}$ (Harghita 2005), or 75.3 $\%_{000}$ (Brasov 2006), suggesting high rates for the WHO goal to eradicate measles by 2010.

In order to eradicate measles by 2010 it well by necessary to: improve access and support for increasing the vaccination coverage to promote prevention and control measures against epidemic outbreak and to extend de surveillance system.[1,3,4]

For the years 2005-2006 there was a large number of measles cases, beyond expectation. That allows us to confirm the epidemic evolution of the disease in Harghita, Brasov and Sibiu counties, extended epidemic being registered for other counties[5,6], or in some European countries.[1,7]

The 2005 epidemy in Harghita County occurred on a mostly unvaccinated population, vaccination coverage being 77.4 %, caused by high receptiveness favorable circumstances for a epidemic to appear. A lots of cases were noticed on gipsy children, and one of the reason of that low vaccination coverage was the vaccine shortage.[2]

It is well known that in case of a vary contagious disease such as measles, the potentially vulnerable cases increase despite the increased vaccination coverage and when the incidence of the illness is minimum in time (epidemics "after honey mouth").[1]

The spreading of measles was firstly caused by discontinuity in providing measles vaccine, needed for the routine vaccine and school campaigns included in The National Programs and Immunization. That situation leaded to a large number of vulnerable cases among the unvaccinated, to which they added the percents of the unvaccinated coming from hard accessible location or the ones not having a family doctors.

For 2006 nosocomial transmission of the disease was a characteristic of that epidemy, according to the reports it is noticeable that in a number 8 counties, a percents of 8.5% out of causes contacted that in a pediatric ward. Definitely this percent is higher but the nosocomial character was not always admitted by the sanitary facilities.[6] Out of the 8 counties that each reported nosocomial transmitted cases it is mention: Mures (5/35 cases= 14.3%), Brasov (42/403 cases= 10.4%), and Sibiu (2/80 cases= 2.6%).

Reaching real vaccination coverage of 95% has become a desirable purpose hard to accomplish taking into consideration that the interest of family doctors in vaccination decreases in time. Their knowledge about stoking method and using a measles vaccine is pour and of course the parents' refusal because of religious and ethnic believes.

CONCLUSION

1. Measles is a catching disease a highly contagious which needs maintaining in extended vaccination coverage (over 95%).
2. The measles epidemic occurrence was predicted ("expected") by the experts several epidemiological criteria being met such as vaccination covering under 95 % in the last three years.
3. The high percentage of vaccinated children coming from hard accessible communities and of those ones not having family doctor.
4. The nosocomical measles occur because of disregarding isolation precautions.

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Role of life stressful events in the onset of depression among young people

A.M. Cupșa, I. Andronic, Florina Matei

Aim of the study – to detect the depressive episodes among young people and to find out the role of psychological stress in the onset of depression at this group of population. Method: 110 subjects with age between 19 and 24 years old were followed up from 15 November to 15 December 2008 using a 35- question inventory for socio-demographic and psychological aspects. At the same time, the presence and the level of depressive symptoms were assed using Zung Self Rated Scale for Depression (ZSRSD). Results – the prevalence of minimal and moderate depression was within the range for this group of population. The factors of psychological stress were the most often pointed out factors for the onset of these episodes. Conclusions: young people are vulnerable specially at psychological stress, having risk for the onset of depression. Zung Self Rated Scale for Depression has proved to be a useful inventory in screening population outside of the Public Health Services registration.
***Key words**: young, depression, Zung, stressful events*

Depression is one of the most concerning health problem worldwide, the lifetime prevalence is about 5.5% for males and – 11.8 % for females.[3] The risk of having a depressive episode during lifetime is about 6% for anyone.[2] Young adults represent a special population in this matter, more than 50% of the depressive disorder case occurring in the second and third decade of life.[1] In 25% of the cases, a stressful life event plays a causal role in the onset of the first depressive episode.[5] Divorce, death of a dear one, unemployment are in the top of the 10 life changing stressful events list.[4] Depression was ranked as the fourth leading cause of disease burden[9], being well known from all studies that lost workdays due to sick-leave, reduced working capacity or death are the most critical and major sources of the cost of affective disorders.[6]

In this context, the early detection of depressive symptomatology among young employees, is an important action to take, not only in prevention of depressive disorders and reducing cost related to illness but in increasing overall quality of life for these individuals.

MATERIAL AND METHOD

From more than 400 subjects included in our study for the PhD thesis entitled „Clinical and epidemiological particular aspects in Depressive Disorders among young adults", we chose a subgroup of 110 workers at the Textile Factory of Craiova. Subjects were aged betwen 19 and 24 years old. The whole group was followed up through 15 November – 15 December 2008, using Zung Self Rated Scale for Depression (ZSRSD) and also a 35 questions inventory regarding socioeconomic, behavioural and psychological aspects. In the framework of this study we found correlations between the scores on ZSRSD and individual stressful events during the last year such as: -history of physical (FT) or psychological trauma (PT),

-history of medical conditions requiring long health care services and medication (MC)

-history of medical conditions affecting one member of the family of the subjects (MCF).

RESULTS AND DISCUSSIONS

In this study, we found out a general prevalence of 34.55 % for the clinically significant depressive symptomatology, value which is within the range, according to the results from other studies on this group of population.[7]

76.36% of the subjects were females and 23.64 % were males (Table I). The average group age was 22.17 years old.

Table I. Gender distribution of the group of study

Females (F)	84	76,36%
Males (M)	26	23,64%
TOTAL	110	100 %

The average score on Zung Self Rated Scale for Depression (ZSRSD) was 46.29 and the middle row point value was 44.

University of Medicine and Pharmacy of Craiova

Table II. Zung Self Rated Scale for Depression scores distribution

Score < 50	72	65, 45%
Score from 50 to 59 (mild depression)	30	27, 27 %
Score from 60 to 69 (moderate depression)	8	7,27%
Scor > 69 (severe depression)	-	-
TOTAL Subjects with Depression	38	34,55%

27.27% of the them (30 subjects) obtained between 50 and 59 points on ZSRSD, score which is significant for a mild depressive episode. 7.27% of them (8 subjects) obtained between 60 and 69 points, score which is significant for a moderate depressive episode according to ZSRSD. None of them obtained scores for a major depressive episode (scores higher than 69). This aspects may find an explanation in the fact that all subject are young, active people, taken into the work field and being physical and psychological assed from time to time so that any major disorder would be guided to the Health Care Services.

The rest of them (64.35%) had scores under the threshold for depression on ZSRSD (<50 points).

The general prevalence of the stressful events for these subjects has the following distribution: history of medical (unspecified) conditions which required prolonged health care, during last year - 34.54%; history of medical (unspecified) condition to one member of the family / one dear person, requiring prolonged health care – 38.18%; history of physical traumatic events during last year – 1.8%; history of psychological stressful events during last year – 4.54% (Table III). From all above, only the psychological stressful events showed a statistical relevant correlation with the onset of a depressive episode ($p = 0.048$).

Table III. General prevalence of stressful life events in the group of study

History of medical (unspecified) conditions (MC)	38 / 110	34,54%
History of medical (unspecified) conditions affecting a family member (MCF)	42 / 110	38,18%
History of physical traumatic events (FT)	2 / 110	1,8%
History of psychological trauma (divorce, loss of a dear one, unemployment) (PT)	5 / 110	4, 54%

History of medical conditions affecting subjects or a close related person (such as a member of the family) and the physical traumatic events in the last 12 months, had no statistical correlations with the onset of a depressive episode (Table IV), though they are included among the first ten life - stressful events[4], having causal potential in the onset of the first depressive episode.[1]

Table IV. Statistical relevance of the stressful life events for depression

MC	16 (42 %)	$p = 0,40395$; $\chi 2 = 0,70$
MCF	12 (31,57%)	$p = 0,4832$; $\chi 2 = 0, 49$
FT	2 (5,26%)	$p = 0, 5919$
PT	5 (13,15%)	$p = 0, 0482$
Total Subjects with Depression	38	

In our study, we followed up young subjects (aged under 24), who had, because of this, much lower probability to experience physical traumatic events and medical condition leading to personal disability or requiring long term health care. On one hand, from data available in this moment, we had no possibility to assay the relations of the subjects within theirs families, the level of emotional involvement and the psychological impact that a medical condition affecting a family member, can produce on one. This detailed analysis can be reached only after a structured clinical assessement such as MINI (Mini International Neuropsychiatric Interview) or SCAN (Schedule for Clinical Assessment in Neuropsychiatry). Further clinical examination need to be taken in the near future.

One the other hand, ZSRSD is proved, in many studies, to have high sensitivity in detecting and evaluating the severity of depression but does not stand for a clinical diagnosis.[8] Finally, high scores on ZSRSD may be explained by other socio-demographic aspects not yet taken into discussion, such as – level of income, marital status, type of family, number of persons in the house, number of siblings, home characteristics. Even though, only in 25 % of cases, the first depressive episode may have an external leading cause[5], for the following ones the play role of an external event is even lower.[1,5]

The psychological traumatic events, not only that were statistically correlated with the onset of depression among these subjects, but pointed the highest scores on ZSRSD.

From a detailed analysis, we found out that subjects which reported «divorce / break – up situation» during last 12 months, also got the highest scores from the group (69.68 points). Reffering to this, we must emphasize the formal boundaries for a depressive episode and the errors which may result from using a self- rated scale. For these subjects with limit - scores, any underestimated answer in the inventory would have led them to another category on ZSRSD (severe depressive episode). That is why, a clinical assessment also needs to be performed. With scores right beneath them were situated the subjects which mentioned psychological traumatic events such as « death / loss o a dear one». «Unemployment» during last year, made a low limit score for mild depressive episode, not much different from the subjects who did not report any stressful event.

CONCLUSIONS

Depression represents one of the most important health problem worldwide, affecting the entire population and having a particular high prevalence

among young adults. These subjects have lesser rates of exposure to physical traumatic events and medical condition leading to disability and showed no statistical correlation with depressive symtomatology. Medical condition affecting a family member, also did not match with the onset of depression at these subjects.

Psychological stressful events such as divorce, loss of a dear one, unemployment proved to play an important role in the onset of depression also at this group of population. Zung Self Rated Scale for Depression proved to be a useful inventory in screening depression among young working adults. The psychological vulnerability of young subjects for stressful events requires active involving in detecting early depressive symptomatology in this population.

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Victims of domestic violence in Romania

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Aim: To obtain a diagnosis regarding the violence among Romanian couples and to warn the qualified Romanian legislative institutions about this public health issue.
Methods: A questionnaire with 77 items was applied to 84 women who were victims of violence. The subjects were selected among the victims of domestic violence who asked some specialized Romanian institutions to help them. In order to appreciate the violence within the family (causes, reasons, solutions), another questionnaire was applied to 33 persons considered to be the witness group. These data were statistically analysed using the SPSS program and the final results were interpreted using a chi square test, a t test and a cluster analysis in two steps.
Results: Domestic violence may occur in all the families irrespective of their socio-economic status, but the phenomenon has greater proportions in the families with a low socio-economic status, families with problems such as the lack of a job, poverty, alcohol drinking. Material difficulties, unemployment generate and maintain stress within the couple. Alcohol drinking combined with domestic issues, alcohol drinking combined with certain personality traits or mental illnesses also generate violence.
Conclusion: International covenants signed by many CEE/FSU countries require signatories to collect statistics on domestic violence. Despite these requirements, data on the prevalence of domestic violence throughout the world is still difficult to obtain
***Key words**: domestic violence, victim, public health*

Violence is a phenomenon which has been clearly defined or delimited neither by science nor by the everyday language. When mass media uses this word, they usually refer to the following aspects of violence: "The intentional use of physical force or power, threatened or actual, against oneself, another person, or against a group or community, that either results in or has a high likelihood of resulting in injury, death, psychological harm, maldevelopment or deprivation."[30]

Wife beating and other forms of physical abuse of women by husbands, especially in developing countries context, have received increased attention in recent years, in our country and elsewhere; available population-based evidence is largely based only on reports of women.[12]

The present article defines "the family violence" as any physical or emotional harmful act which happens among the members of a family. The abuse within a family can have many aspects: verbal abuse, refusing any access to financial resources, isolation from friends and family, threats and attacks which sometimes can cause the death of one of the partners. And yet, in spite of the fact that woman has been most frequently considered the victim of family violence, as a result of recent researches, it has been formed that, in fact, the number of abused men is big enough. The experts who have studied this problem agree that violence is a widespread phenomenon, more often found that it appears in surveys, because some facts are not reported to police or hospitals.

The problems analysed in this article refer to (1) the correlation between alcohol consumption and family violence, (2) the anthropologic profile of the aggressor and his victim and (3) post-violence attitudes within a couple.

METHODOLOGY

The "Francisc I. Rainer" Anthropology Institute, Romanian Academy, accomplished the Grant "Domestic violence – an anthropological violence" in 2003-2004. A questionnaire with 77 items was applied to 84 women who were victims of violence. The subjects were selected among the victims of the domestic violence who asked some Romanian institutions of the kind to help them. In order to appreciate the violence within the family (causes, reasons, solutions), another questionnaire was applied to 33 persons considered to be the witness group. These data were statistically analysed using the SPSS program and the final results were interpreted using chi square test, t test and cluster analysis in two steps.

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The research had two purposes: (1) to obtain a diagnosis regarding the violence; (2) to warn the qualified legislative Romanian institutions, by means of scientific reports and articles published in specialty journals, in order to offer a temporary shelter to women who were victims of the domestic violence, women who really represent a problem in our country.

Some of the evaluated items were:

In what way do you think family is involved in the violent pattern ?

For how much do you think that the following causes have induced the violent behavior of the subject ?

Wich is your answer/attitude to the abuse suffered ?

Can you see a possibility of changing the violent behavior, a chance for reconciliation ?

In the whole, the research aimed at offering answers to some general and special objectives such as: identification and explanation of social and individual causes that lead to family violence; pointing out the main risk factors which generate violence within the family; establishing of some categories of violence and post-violence attitudes and so on.

RESULTS

1. Alcohol consumption and family violence

The question referring to the frequency of certain deviations from the marital behaviour had the following results: in the victim's birth family, the issues which appear "often" and "very often" are (in a decreasing order) the deficient administration of the budget and the lacks (33.3%), the conflicts (27.4%), the deficient implications in raising the children and the lack of interest (25%), alcohol drinking (20.2%). But these aspects are not characteristic to the victims' birth families because the frequency in which they appear "never" and "very rare" is bigger than "often" and "very often". It is possible that the situation is real or the self-appreciation of the victim's birth family is more indulgent and/or the limits of the various degrees of violence are not well enough defined.

According to the victim's appreciation, it was found out that in the aggressor's birth family the problems which appear "often" and "very often" are (in a decreasing order) conflicts (48.8%), alcohol consumption (46.5%), the deficient administration of the budget and the lacks (36.9%), fights among the family members (27.3%). So we notice a different hierarchy in the aggressor's birth family.

We point out the fact that in the aggressor's birth family the conflicts and the alcohol consumption change into characteristics for this family, because the frequency which they appear "often" and "very often" is almost double compared to "never" and "very rare" (Table I).

Pearson chi-square test shows a significant difference between the frequency of alcohol ingestion in the victim's birth family and the aggressor's birth family ($p<0.01$). By imitation and identification processes, the child assimilates certain behavioural patterns which will reproduce in his own family and society.

Among the causes pointed out by the victim as generating a violent behaviour, alcohol consumption is on the first place; on the second and the third places, with almost equal values, there are the material difficulties and the entourage. A percentage of 85.8 of the victims report that alcohol drinking caused "much" and "very much" the aggressor's violent behaviour (Table II).

In order to obtain as much information as possible regarding the causes of violence, we used an open question, to let the victims freely express their opinion. Alcohol drinking (51.8%) and material difficulties (22.2%) were considered as being the main obstacles in a normal family life.

Table I. Alcohol abuse in victim's and agressor's family of origine

Alcohol abuse in victim/agressor's family of origine	Victims %	Agressor %
Never	50.6	21.4
Rarely	28.9	32.1
Often	20.5	46.5
Total	100	100

Table II. Causes for the violent behaviour of the agressor

Causes induced by the violent behaviour of agressor's family	Not at all	Little	A lot	No answer
Alcohol abuse	8.3	5.9	85.8	0
Agressor's lack of devotion	69.1	13.1	17.8	0
Victim's lack of devotion	87	5.9	5.9	1.2
Sexual and emotional dissatisfaction	57.1	19.1	20.2	3.6
Financial difficulties	21.4	9.5	69.1	0
Entourage	15.5	15.5	67.8	1.2
Working place dissatisfaction	46.4	21.4	32.2	0
Family bad education	32.1	22.6	44.1	1.2
Low level of education	41.7	29.7	28.6	0
Other causes	78.6	1.2	2.4	17.8

Excessive alcohol drinking causes brain problems which affect the ability of fulfilling daily work, generate the loss of memorizing and the diminishing of cognitive capacities; it also affects the vestibular analyser, the hepatic cell, etc. Alcohol abuse is not a cause in itself, but a condition which hastens aggression, provoking exaggerating reactions, confusion in thinking and the lack of perception of the consequences of the violent behaviour. Most of the conflicts are caused by the alcohol drinking.

2. The anthropological profile of the aggressor and of the victim

The appreciation of the victim's and the aggressor's anthropological profile was made according to the age and constitutional type criteria. The following body dimensions were taken into account: weight, height, breadth of the shoulders and pelvis, circumference of the waist, hips and thorax, calculating different values relevant for anthropological comparisons. In this article we analyze weight, height, Body Mass Index (BMI) and the afferent correlations victim-aggressor.

In the anthropological chapter the research had mostly an investigating character and, thus, the following assertions are not representative, they mainly refer to the studied group. Regarding the height, we point out that, in the studied group, the number of tall aggressors is approximately four times bigger than the number of the victims belonging to the same height category (20.2% vs. 4.8%). Although this aspect is not representative, we can say there is the tendency women who are shorter than their male partners are more easily exposed to the risk of being aggressed within the couple.

Though the increase in weight (in absolute value) is bigger for women, we show (Table III) that the number of the overweight aggressors and those with first degree of obesity is bigger than the number of women belonging to these two weight categories (41.7% vs. 23.8%).

Cluster analysis in two steps based on two variables. The Two Steps Cluster Analysis has the possibility to point out the significant or non-significant contribution of each analysed variable to the formation of natural groups. We have analysed the BMI both of the victims and the aggressors. Of all our data, 92.9% are validated and 7.1% are excluded because of the lack of answers which were declared missing (table IV).

As the table shows, the analysis identifies three specific clusters including 45 cases in cluster 1, 24 cases in cluster 2 and 9 cases in cluster 3.

In table V, this analysis presents the central values (centroids) of the clusters (cluster 1, 2, 3 and combined cluster) expressed through the average value and the standard deviation of each analysed variable. The average values and the standard deviations for all the analysed cases are presented also for the combined cluster.

In cluster 1 (the best represented), the average value of the index is 22.55 for the victim and 22.95 for the aggressor. Having almost the same value, they are below the combined general average (23.80 for the victim, 25.28 for the aggressor). In 53.6% cases, the victim and the aggressor have almost the same value for

Table III. Quételet index related to victims and agressors

Quetelet index	Agressor %	Victim %
Underweight < 18,49	0	4.8
Between 18,5-19,99	1.2	8.3
Normal weight (20-21.99)	14.3	16.7
Between 22-24,99*	39.3	39.3
Overweight (25-29.99)	29.8	16.7
Obesity I degree (30-39,99)	11.9	7.1
Obesity II degree >=40	0	1.2
No answers	3.5	5.9
Total	100	100

*18,5-24,99 normal scale

Source for Quételet Index : Utilisation et interprétation de l'anthropométrie, Rapport d'un comité OMS d'experts, Organisation mondiale de la Santé, Genéve, 1995, p. 496.

Table IV. Body mass index distribution in cluster

Cluster distribution	No.	% of Combined	% of Total
1	45	57.7%	53.6%
2	24	30.8%	28.6%
3	9	11.5%	10.7%
Combined	78	100.0%	92.9%
Excluded Cases	6	-	7.1%
Total	84	-	100.0%

Table V. Quételet Index median for clusters formed

Centroids		Quételet Index for victim		Quételet Index for agressor	
		Mean	Std. Deviation	Mean	Std. Deviation
Cluster	1	22.5588	2.89241	22.9451	1.28559
	2	22.7360	2.46419	27.5203	1.78799
	3	32,8842	4.40918	31.0610	2.37694
	Combined	23.8047	4.41735	25.2893	3.33809

BMI. The analysis of the correlation between the values of the two BMI indicates a Pearson coefficient r=0.443 at á=0.001. This correlation is not very strong, but it is significant; it could mean these couples victim-aggressor were naturally formed between persons with close physical characteristics.

In cluster 2 (28.6% cases), the average value of BMI is 22.73 for the victim and 27.52 for the aggressor. The value of the index for the aggressor is almost 5 units bigger than that of the victim. The value of the index of the victim is below the general average, but the aggressor's index is significantly bigger than the general average for the aggressors.

In cluster 3 (10.7% cases), BMI for both victims (32.88) and aggressors (31.06) are above the general averages. Both victim and aggressor can be considered obese persons.

The general average validated for all cases shows an aggressor with a BMI of 25.28 who aggresses a victim with a lower BMI (23.80).

3. Post-violence attitude

After they have been aggressed, most of the victims (40.5%) feel trapped and shocked, but they do not see any possibility to change things. The other answers were distributed approximately equal (about 29.8%) in the category of fatalists, who blame on the fate ("it was meant to be"), and of those who declare that they are decided not to accept this behavior anymore.

Applying the chi square test, it was pointed out the significant correlation ($p < 0.05$) between the attitude against the suffered aggression and the income level. More than three quarters of the victims with a low level or with no income declared "it was meant to be" or "I am shocked, but I cannot do anything". We draw attention that most of the victims who declared "I will not take it anymore" have an average income.

Being asked if they saw any chance of change or reconciliation, the victims generally were pessimistic about this chance. The optimistic attitude of the victim (the appreciation of some possibilities of change) is also significantly correlated to the income level ($p < 0.05$), but not to the victim's education and profession. We point out the bigger percent of pessimists among the victims with a minimum school training comparing to those with medium school training; the greatest number of pessimists is recorded among the jobless women.

The victims who have no job and are decided not to accept any aggression anymore are the fewest. So we remark that the number of victims who do not accept any aggression tends to increase at the same time with the rise of their professional status.

DISCUSSION

The family that forms the scene for family violence manifestation is becoming less transparent and less open to the immediate social background – extended family, neighbour, friends, colleagues. It is obvious the social isolation of this family. The violent husband does not want his wife to have social relationship within which to be able to confess her suffering and possibly to get help.

In Europe right now the statistics of male violence against female partners are terrible. For European women aged 16-44 violence in the home is the primary cause of injury and death, more lethal than road accidents and cancer. Between 25% and 50% of women are victims of this violence. In Portugal 52.8% of women say that they have been violently treated by their husbands or partners. In Germany almost 300 women a year - or three women every four days - are killed by men with whom they used to live. In Britain one woman dies in similar circumstances every three days. In Spain it is one every four days. In France six women die this way every month: 33% of them are knifed, 33% shot, 20% strangled and 10% beaten.[26] In the 15 member states of the European Union (before enlargement to 25), more than 600 women die every year because of sexist brutality in the family.[26]

According to the Family Violence Prevention Fund (FVPF), one in every three women in the world has experienced sexual, physical, emotional or other abuse in her lifetime. The World Health Organization (WHO) reports that in forty-eight surveys from around the world, 10-69% of women stated that they had been physically assaulted by an intimate partner at some point in their lives. The WHO also reports that studies from a range of countries show that 40-70% of female murder victims were killed by an intimate partner.[3,10,33]

The Astra Network has reported that "29% of women in Romania, 22% in Russia and 21% in Ukraine, reported experience of spousal physical abuse" and that over 42% of all married and cohabiting women in Lithuania "reported that they have been victims of physical or sexual violence or threats of violence by their present partner," although only 10.6% of the Lithuanian respondents "reported the most serious incident to the police." In Romania, 29% of adult women in a national survey reported having been physically assaulted by an intimate partner.[32]

Regarding our objectives:

Most of the conflicts are caused by alcohol drinking. Alcohol drinking in the aggressor's birth family is statistically significant higher than in the

victim's birth family. By imitating and identifying, children assimilate certain behaviour patterns they will reproduce in their own family and in society.

Alcohol drinking is a social problem, a public health problem, especially in the case of Romania which is a country with traditions in making alcoholic drinks at home, and for this reason part of the production and of the consumption remains unknown.[7,8,19,27]

Individual factors and factors related to the family and to the friends' entourage are significant indicators for the abusive alcohol drinking. The aggressive behaviour and alcohol drinking have common factors of risk. Significant predictive factors for alcohol drinking are: being male, having frustrations, having friends who get drunk at least once a week, having brothers/partner who get drunk, being the reason for physical aggression, being dissatisfied with the partner relationship.[5,9,27]

When both parents have problems with alcohol drinking, there is a high risk of growth and behaviour problems for children. Because the family has financial troubles caused by not having a job, arguments appear between parents, arguments which frequently degenerate into physical violence. Alongside alcohol abuse, parents can have co-morbid affections (such as depression) which can also disturb children's growth. Even we do not pretend our study is representative, we can affirm the couple victim-aggressor was naturally made between persons with similar physical traits and anthropological profiles. The prevalence of tall aggressors is approximately four times bigger than that of the victims belonging to the same category. There is the tendency that women who are shorter than their male partners are more easily exposed to the risk of aggression within the couple.

There are studies which pointed out that family violence is more frequent among families with small incomes and low level of training, mainly for two reasons: (1) favoured families do not speak about the violent situations happened inside; (2) unfavoured families use violence as a means of solving their frustrations.[1,13,14,23,28]

Our study emphasyses the fact that the economical inequality of the partners and the economical authority of men are predictable factors of the abuse of the wife. Other authors reached similar conclusions.[15,20,21,24,25] Thus the non-acceptance attitude is statistically significant less among victims without income or with low income than among victims with average or over-average income.

In our study group the relationship between the school training level and the attitude towards aggression/the appreciation of their chance to reconciliation is not statistically significant. However the victims with a lower lever of training tend to accept domestic violence; the decision of unacceptability tends to increase at the same time with the increase of the professional status. We can say that the social recognition given by a diploma or a profession is less important than the financial situation. The economic independence encourages the victim to break up with the partner or even divorce. However, the victims with a lower level of training tend to accept domestic violence and the tendency of unacceptability increases at the same time with the increase of the professional status.[11,22]

Domestic violence can manifest in all the families irrespective of their socio-economical status, but the phenomenon has greater proportions in the families with low socio-economical status, families with problems such as the lack of a job, poverty, alcohol drinking. The victims in these families, generally financial dependent, have smaller resources to avoid aggression, opinion sustained by other studies, too.[16,17,18]

CONCLUSIONS

Material difficulties, unemployment generate and maintain stress within the couple. Then there are couples where only husbands have control over the family budget. Alcohol drinking combined with domestic issues, alcohol drinking combined with certain personality traits or mental illnesses generate violence.

Statistics relating to the prevalence of domestic violence are critical to any advocacy effort. Statistics can help document the need for certain programs or raise public awareness of the extent of the problem. International covenants signed by many CEE/FSU countries require signatories to collect statistics on domestic violence. For example, the Declaration on the Elimination of Violence Against Women recommends that states parties "promote research, collect data and compile statistics, especially concerning domestic violence, relating to the prevalence of different forms of violence against women and encourage research on the causes, nature, seriousness and consequences of violence against women and on the effectiveness of measures implemented to prevent and redress violence against women." Despite these requirements, statistical information on the prevalence of domestic violence throughout the world or in the CEE/FSU region is still difficult to obtain.[29,31,33]

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Study of risk factors for gastro-intestinal diseases influenced by life-style conditions and dietary practices in children

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Peptic ulcer disease represents one of the most important and most frequent gastrointestinal pathological conditions in our century with multiple etiologies. Besides etiological factors a lot of risk factors can be considered important contributing to the development of this disease. There are a number of psychosocial factors which are important for ulcerogenesis, besides Helicobacter pylori infection or nonsteroid antiinflamatory drug use. The aim of our study was to evaluate life-style conditions and dietary practices of children, concentrating on risk factors for gastric and duodenal ulcer. We made our study using a questionnaire with 27 questions in a group of 210 children with their age ranking from 11 to19 . From the point of view of risk factors we considered that the majority of children are exposed to significant stress because of school. Children begin to consume alcohol, coffee and smoking at a very young age (12-14 years), the majority of them doesn't have normal body weight (are overweight or underweight). Unhealthy dietary practices together with other risk factors influence the development of peptic ulcer disease. Health educational programs, reducing of stress and risk factors could decrease the risk of peptic ulcer pathology development.

Key words: peptic ulcer disease, risk factors, dietary practices, health education

The importance of studying psychosocial risk factors, mainly life-style and alimentary factors in the pathogenesis of peptic ulcer disease (PUD) (gastric and duodenal ulcer) is represented by the fact that in our century these are the most frequently diagnosed gastrointestinal clinical cases, with multiple etiology. The main complications, for example upper gastrointestinal bleeding is the most common gastroenterological emergency.[17] The prevalence of PUD is 21-29% in men and 11-18% in women population.[7,8,13,18]

It is well-known that PUD can be best understood as an imbalance between factors that protect the gastric mucosa and aggressive factors for gastric mucosa. This is the main mechanism in the pathophysiology of PUD. Creating an optimal peptic healing environment requires facilitating the protective factors and reducing and/or eliminating aggressive factors. Aggressive factors include acid-peptic activity, impaired gastroduodenal blood flow, inflammation, Helicobacter pylori infection, and nonsteroid anti-inflammatory drug (NSAID) use. In spite of these there are many different factors that contribute to the imbalance between protective and aggressive factors, which are less studied in our century, including stress, environmental, dietary and life-style factors.[13] This is because the identification of Helicobacter pylori (H. pylori) in the 1980s significantly shifted the focus of PUD to an infectious cause and management, overshadowing other factors, considering them not so important.[15] H. pylori is often emphasized in regards as etiology of PUD and so the treatment is axed on eradication of H. pylori. Although H. pylori has been suggested to play a protective role in infant diarrhea and esophageal disease the association with PUD is well documented.[15,19] H. pylori is not the only cause of PUD, because only 20% of all persons infected ever develop an ulcer (whereas 70% of individuals with gastric ulcers and 70% to 90% of duodenal ulcer patients have H. pylori infection).[11] Thus, H. pylori may be considered a significant cofactor in the etiology of PUD, and interventions that prevent, suppress, or eradicate H. pylori are likely to be beneficial.

There is also considerable emphasis on the relationship of NSAID use and PUD. Despite the fact that both have a significant role in the formation of ulcer, they still are not the causative ethiological agent for PUD. Although symptoms of PUD are seen in up to 20% of individuals taking them, most persons using NSAIDs never develop PUD; in addition, 10% of non-NSAID–related peptic ulcers have no H. pylori infection. We can conclude that these two

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Table I. Body-mass Index groups

<18	malnutrition
18-19,9	underweight
20-24,9	normal weight
25-29,9	overweight
30-34,9	I. obesity
35-40	II. obesity
>40	III. obesity

Table II. Repartisation of children in groups due to dietary practices

Groups	Total		Acceptable		Unhealthy		Healthy	
	Children nr.	%	Children nr.	%	Children nr.	%	Children nr.	%
Village	83	100%	70	84,3%	3	3,6%	10	12,1%
Town	127	100%	106	83,4%	9	7,1%	12	9,4%
Total	210	100%	176	83,8%	12	5,7%	22	10,5%

etiopathogenetic factors cannot be considered as only causes of peptic ulcer disease.[14]

Many factors play a role in increasing the risk for PUD, including stress, low socioeconomic status, tobacco use, sleep deprivation, nutritional deficiencies and practices, alcohol abuse, acid hypersecretory states (Zollinger-Ellison syndrome and possible hereditary states), localized vascular insufficiency, and having a spouse or partner with PUD.[9,10] Epidemiologic studies show a decreased risk correlated with good dietary practices and increased physical activity.[4,6,16] Psychosocial factors are recognized to contribute to 30% to 65% of peptic ulcers whether due to H. pylori, NSAIDs, or neither.[11,12] Most striking are epidemiologic evidence supporting increased risk of PUD following significant traumatic events (e.g., the London air raids of World War II and earthquakes).[5]

Start out from this point of view, the aim of our study was to evaluate dietary practices and life-style conditions of children, concentrating on risk factors for gastric and duodenal peptic ulcer disease in the interest to initiate a preventive program in schools.

MATERIALS AND METHODS

We used a questionnaire for our study in a group of 210 children between ages 11-19, studying in primary and secondary schools. Our questionnaire contained 27 questions. These questions were specially formulated to collect information about life-style and alimentary practices of children. So that our questions referred to the dietary practices, smoking, alcohol abuse, stress and regulate physical activity. For the evaluation of results we used a scoring system.

We calculated for every child the Body-mass Index (BMI) after the following formula: $BMI=W(kg)/H^2(m)$, where W=body weight, H=height. Using BMI results we classified children in different groups.

RESULTS

The dietary practices of the 83 (100%) children from rural environment were acceptable at 70 children (84,3%), unhealthy at 3 children (3,6%) and healthy at 10 children (12,1%). Comparative with this, dietary practices of the 127 (100%) children living in urban environment were acceptable at 106 children (83,4%), unhealthy at 9 children (7,1%) and healthy at 12 children (9,4%).

On the basis of BMI we can distinguish 88 (41,9%) normal weight children, 108 (51,5%) underweight children and 14 (6,6%) overweight children.

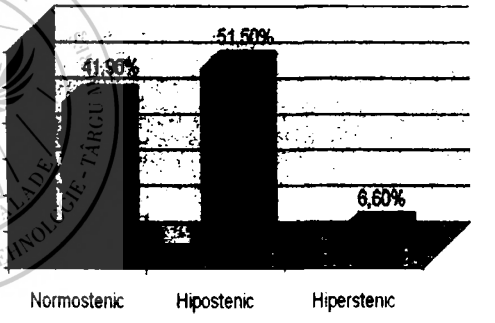

Figure 1. Repartisation of children due to Body-mass Index

The children in a proportion of 13,3% never have breakfast, and more than 50% of them have healthy lunch once a day, but the others consume fast-foods or sweets. 34% of the children consume regularly sweets and 9% consume only carbonated sweet drinks. Despite the fact that our questionnaire was anonymous, in our opinion the answers to the questions about smoking, or alcohol and coffee consumption were not real. Only 3,8% of the questioned children smoke, 4,28% consume coffee every day and 7,6% consume alcohol every week. Surprisingly we found that 2 primary school children consume alcohol every day and 2,8% of them want to try out different drugs. Most of the children are exposed to significant stress factors mainly because of school.

DISCUSSIONS

PUD maintains a multifactorial etiology with diverse risk factors that include both endogenous and exogenous factors, both physical and psychosocial.[13]

These factors may work independently but most likely they work in concert to unbalance the homeostasis of the gastro-duodenal environment. The diversity of PUD risk and protective factors makes the prevention and treatment ideal for an integrative approach.[11,12]

Our results reflect clearly life-style and alimentary practices of children from primary schools, between ages 11-19, these are similarly in those from urban and rural environment. Surprising is the fact that unhealthy habits of our century have their effects not only on adult's life but on children's life, too. Most of them go to school without having breakfast; they do not have lunch and prefer sweets and fast-foods. Fortunately mineral and vitamin needs are insured from vegetables and fruits. Unfortunately children prefer carbonated sweet drinks instead of mineral water or natural juice. Among children from secondary school consuming coffee is very wide-spread. Many children start their day with a coffee instead of having breakfast, or consuming milk, simply things that are so important for the healthy growing of children's. One of the worst disadvantages of the development of computer-techniques is that most of the children spend too many hours per day in front of computers instead of going out to play or doing physical exercises on a regular base. The alcohol abuse and smoking is very popular in the 21st century which is wide-spread mostly in secondary schools, but younger children are curios to try these out too, many of them already smoke (at the age of 12-14), some of them are consuming alcohol once a week or every day. Childrens' curiosity does not have limits, and many of them want to try out different drugs, without thinking about the consequences.

CONCLUSIONS

From the children we asked 58,10% doesn't have required body weight (they are underweight or overweight), fact that is the consequence of unhealthy dietary practices. The deviation of body weight from the normal can be considered as a risk factor for a lot of pathological conditions, because obesity or malnutrition have negative effects on human immune resistance.

In conclusion we can say that the majority of children in our century are exposed to significant stress, have low socioeconomic status and deficient nutritional conditions because of unhealthy dietary practices which include consume of fast-foods, sweets, coffee or skipping breakfast. Very young children begin to smoke (at the age of 12-14 years) and the abuse of alcohol can be considered to be a problem already among children. The development of computer-techniques of our century has effects on children's life. They stay too many hours in front of the computer instead of playing games or doing physical exercises on a regular base.

In the first part of this article we quoted data from the scientific literature about risk factors for gastrointestinal illnesses. All of our results can be considered to be risk factors for a lot of diseases, containing gastric and duodenal ulcers, too. We can conclude, based on our results, that the introduction of some prevention programs and health education in schools would be useful for the prevention of gastrointestinal diseases.

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Current trends in rectal cancer surgery – the experience of the 1st Surgical Clinic, Târgu Mureș

O.C. Diac[1], C. Copotoiu[2]

The modern treatment of the rectal cancer involves the mesorectal excision, no matter the type of operation, rectal amputation or saving sphincter operation. TME is considered the golden rule in rectal cancer surgery for most English and American surgeons in all the radical operations. The aim of this study is to analyze the rectal cancer cases registered in Surgery Clinic I between June 2007- December 2008. Were recorded 78 cases; we followed the location of the tumor, mesorectal and distant metastasis, local complications and the type of the operation performed; also the postoperatory complications. We studied the way that preoperatory radiotherapy influenced the possibility to perform saving sphincter operations.
***Key words**: rectal cancer, mesorectal excision, limph nodes metastasis*

Colorectal cancer is the third cancer as global frequency and the main cause of morbidity and mortality by cancer in Europe and USA. Survival at 5 years in patients with colorectal cancer is less than 50% due to increased risk of local recurrence and distant metastasis (this being produced even under conditions of resections considered curative.[2] Of the total colorectal cancers 45% are located at recto-sigmoideum junction and rectum.[13]

Surgical treatment of rectal cancer has undergone a qualitative leap, a default rate of survival at 5 years better in the last 20 years with the definition of the concept of mezorectum.[8]

MATERIAL AND METHOD

Lot in the study includes a number of 78 patients admitted and treated in the surgery clinic I, during June 2007-December 2008. Of the total number of patients are 51 men and 27 women, with a rate de1, 8 / 1 the average age was 64 years.

In the studied group we followed the frequency of types of surgery performed in relation to different locations of rectal neoplasia (1/3 upper, 1/3 middle și1/ 3 lower), presence of macroscopic ganglion metastasis in mezorectum, locoregional penetration of tumor and spreading distance and how they influence the operative conduct. For documentation have been used in descriptions of clinical observation of patients and operator protocols were introduced in a data base MS Access and processed to MS Excel.

RESULTS

In the period studied were 78 reported cases of rectal cancer operated, of which 51 men and 27 women with age between 34 and 84 years (mean age 64 years). In the case tracked 56 patients were chronically hospitalized, 22 patients were hospitalized and operate in conditions of emergency or urgent deferred. Of the total cases tumor location was reported at the recto sigmoideum junction in 21.3% (n = 19), in 1 / 3 upper rectal 40% (36 cases), in 1 / 3 medium 12.3% (n = 11) and 1 / 3 lower rectum presented 19 cases (21.3%). In 4 cases there was no stated tumor and in one case was anorectal and sigmoidian synchronous cancer, another case presented a perforated neo gastric and lower rectal tumor.

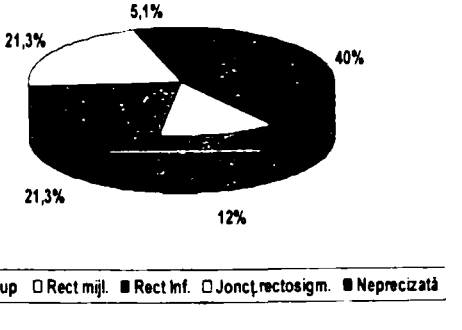

Figure 1. Location of tumor

Intraoperatory during exploration of peritoneal cavity was found presence of tumor ganglion metastasis of mezorectum in 9 cases macroscopic

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revealed visual and palpable after resection of the preparation specimen, liver metastases were revealed in 8 patients and epiploic metastases in 2 cases. Tumor penetration into neighboring anatomic structures was recorded in 14 cases, 1 case showing a perforated upper rectal tumor along with peritoneal carcinomatosis. During the period studied there was a rectal tumor recurrence after Dixon operation made back in several years.

Table I.Tumoral dissemination

Tumor disemination	Number of cases	%
Liver metastasis	8	10,25%
Ganglion metastasis	9	11,53%
Epiploic metastsis	2	2,56%
Peritoneal carcinomatosis	1	1,28%
Neighborig organs	14	17,94%

Patients of studied lot were performed l abdomino perineal rectum- amputation Miles for 16 cases, anterior rectal resection Dixon in 39 cases, of which 5 cases has been practiced a colo-rectum low anastomosis l, one of them by mechanical inosculate with circular stapler. I Hartmann operation was performed on 10 patients, the operation Mausell-Weill was charged in a case. In all cases in which it was possible to conduct a radical operation has been performed total excision of mezorectum according to principles established by Heald. For tumors of rectosigmoideum junction and rectal or higher where it was possible to conduct a colorectal anastomosis it s been practiced mezorectum partial excision, respecting the same principles of oncological surgery. Colostomy of liberation Maydl was the only surgical solution for 6 patients, and tumor biopsy was performed in 5 patients in order to determine histopathological diagnosis and the possibility of retraining tumor after radiotherapeutic preoperatory treatment. With intention of converting the tumor in one case was done tumor citoreduction along with colostomy.

Table II. Types of surgery practiced in rectal cancer

Operation type	Number of cases	%
Milles amputation	16	20,51%
Dixon resection	39	50%
Hartmen resection	10	12,82%
Mausell-Weill operation	1	1,28%
Mayidl colostomy	7	8,97%
Biopsy	5	6,41%
Tumor citoreduction	1	1,28%
Exploring lapharotomy	1	1,28%

Preoperatory Radio therapeutic treatment has followed 14 patients (18%), surgical intervention being performed 4-6 weeks after this. In agreement with multiple international studies is required by this radiotherapy a range of preoperatory leisure in which primary tumor cells and metastasis are devitalized and tumor produces conversion.[2,10] In these patients it was possible to conduct Miles operation in 8 cases (10.25%) for lower rectal tumors located in organs or penetrating cities (prostate, uterus). Dixon operation was performed in 4 patients (5.12%) irradiated preoperatory who had tumors in 1 / 3 top and middle, and Maunsell-Weill surgery was performed in one case (1.2%) with middle rectal neo and 1 patient refused surgery. Because the presenting of patients in advanced disease (Dukes C, D) was necessary in many cases the use of complex operations, in which were made partial or total resections of abdominal organs (uterus, annexes, bladder). Liver metastasis was treated by metastasectomy, a case was charged with concomitant rectal surgery regulated left lobotomy; another case has received enlarged left hepatectomy, anastomotic colorectal fistula was recorded in one case (2.5% of the 39 operations Dixon), which imposed reintervention, so practiced protection colostomy type Maydl on transverse colon, subsequent evolution is favorable.

DISCUSSIONS AND CONCLUSIONS

Surgery for cancer of the rectum known in recent decades due important progress on the one hand a better knowledge of rectum anatomy[1,7], on the other hand the progress of operator techniques, and application of protocols for radio chemotherapeutic treatment pre-and post- operator.[2,3]

If in the past rectum amputation surgery was considered to preserve best the oncological principles, current anatomical redefinition has allowed carrying out operations that preserve the sphincterian system improving the postoperatory comfort of the patient.[12,18] Treatment of rectum cancer has as an oncologic objective removal of all the primary tumor with the lymphatic drainage territory, and as functional objective keeping the sphincterian system, where the tumor allows, and anatomical preservation of urogenital system function.[1,5] Prevention of complications of sexual dynamics and urinary dysfunctions are closely related to operative technique.[9] Thus, Heald points out to sharp dissection performed to plan between rectal fascia propria and visceral parietal fascia; this plan Heald called "the sacred" is formed of a very thin layer of lax tissue relatively avascular whose compliance during dissection meets the oncologic objective and the functional, of intact storage of genitori-urinary function.[7]

As has accumulated experience in conducting technical TME the incidence of colon-rectal anastomoses fistulas decreased which originally had a high incidence rate. So from 17-20% as recorded in the early years of mesorectum excision, currently recorded, complied with multiple international studies, a anastomosis fistulas rate of 5% or even below this value.[12,16]

Preoperatory radio therapy has a local recurrence rate from 27% to 11% and improved survival to a distance from 48% to 58%.[10]

In conclusion, surgical treatment is the main therapeutic method and the only curative visa, but the radio pre-and post-chemotherapy reduced the number local recurrences and improved survival remote.

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Timing of operation and selection of surgical procedure for variceal hemorrhage in portal hypertension

C. Molnar[1], C. Copotoiu[1], G. Serac[2], A. Panţîru[1], C. Nicolescu[2], C Rosca[1]

Many surgical procedures, including portal-systemic shunts and nonshunsting operation, have been proposed for patients who bleed from esophagogastric varices. The urgency of surgical operation, status of hepatic hemodynamics and experience of the surgeon appear to be the most important factor to consider in selecting the appropiate operation for patient. The timing of the operation with aspect to the acute bleeding episode remains controversial. Consevative methods for temporarly controlling hemorrhage are the first choice if these are available. In our current practice, surgery must „resolve" the dramatic complication, due to variceal bleeding. Selectiv portal-systemic shunt (distal spleno-renal shunt) and nonshunting procedures (azygo-portal disconections) are probably the only procedures that preserve hepatic portal perfusion and this are the less frequently complicated by postoperative encephalopathy.

***Key words**: timing of operations, selection of surgical procedures, portal-systemic shunt, nonshunting operations*

The most dramatic complication of portal hypertesion is hemorrhage from gastroseophageal varices. Althrough only a third of patients with varices ever bleed, once hemorrahage occurs the likelihood or recurrence exceeds 70 percent.[12,5,15] Mortality due to this complicatin has been reported to be from 22 percent[4] to 84 percent[10] and appeart to be dependent on both the severity of the liver disease and the effectiveness of the measures used to control hemorrhage.

The pacients with gastroesophageal variceal bleeding must be admitted in intesive care units. Several nonsurgical therapies, including vasopressin (Pitressin) infusion[7,2] balloon tamponade[1,11] transhepatic embolization of the coronary vein and endoscopic variceal sclerosis[3,14,9] have been effective to variable degrees in controlling acute variceal bleeding and confer long-term protection against recurrence.

MATERIAL AND METHODS

In our conditions, the limited accesibility of these therapeutical measurements, makes surgeons „responsale" for controlling acute variceal bleeding. Multiple surgical strategies have been devised, each with the hope that it would provide the definitive answer to this complex problem: safe life of patient.

The purpose of this review is not to make an exhaustive descripion of the surgical arsenal. Some of these procedures have been „imaginated" sice intraoperative, because frequent surgeons can't assist passive to patient exanquination. This study relieve the importance of well choice in timing of operations and a justified selection of surgical procedure.

These must be adapted to the pacient not to the „clinical case". None of the srugical operations is „ideal"; however, after 32 years of experience in portal hypertension surgery in First Surgical Clinic from Targu Mures, we can give some advices for the practiciners.

From 1st January 1977 to 28th February 2009, we addmited 496 cases with upper digestive bleeding due to gastro esophageal varices and portal hypertension.

From all patient 207 were operated, 124 performed nonshunt operation with a 40,5% mortality rate, and 83 shunt procedures with 21,68% mortality rate.

Nonshunting operation have a high mortality in emergency (70% versus 18,7%) but these were the procedures preferred in urgency, sometime in acute bleeding episode.

Shunting procedures were preferred in elective cases, less important mortality in selective porto systemic shunts

RESULTS

Postoperative morbidity was significant (15%) local complications where recurrence of hemorrhage was most important, and 28,5% general complication, portal encephalopathy, hepato-renal failure was the main pathology.

Postoperative mortality shows that in emergency was more frequently (64,17% versus 19,28%.

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In emergency nosshunting procedures have a low mortality (18,75% versus 20,27%) but in elective cases is higher (69% versus 33,3%).

DISCUSSION AND CONCLUSIONS

Operations for variceal hemorrhage can be done at the following times with respect to the acute bleeding episode:

1. prophylactically, before hemorrhage has occurred
2. electively, at some period after bleeding has been controlled by no surgical methods
3. urgently, during or shortly after an acute variceal hemorrhage.

Prophylactic portal-systemic shunts are not indicated.[11,6,3] Proponents of elective surgery base their argument on the well-established relationship between status of liver function at the time or surgery and surgical mortality. The elective surgical approach is based on the premise that effective non surgical techniques for control of variceal hemorrhage are available. Because of excessive mortality ranging from 20 percent to 73 percent following emergency surgery[3] there are few remaining advocates of this approach to control of acute variceal bleeding.

The proponents of emergency surgery agree that the non surgical methods are generally ineffective and result in higher mortality than non active emergency surgical intervention in these poor risks, acutely bleeding patients.[11]

Selection of surgical procedure is influenced from 3 factors:

1. clinical situation
2. status of hepatic homodynamic
3. experience and skill of the surgeon

However we have not a surgical procedure means to be "enough" and "safely"; portal hypertension and surgical content with large experience shows that three are the most benefic shunting operations (end-to-side portocaval shunt, side-to-side shunts, distal splenorenal shunt) and nonshunting procedures (azygoportal disconnections).

The main clinical factor that bears on selection of the operative approach is the presence or absence of ascites. Ascites develop in most patients with cirrhosis following resuscitation from major variceal hemorrhage. If the ascites resolves with consecutive measures that surgical procedures that would otherwise be appropriate for that patient should be selected. When ascites in intractable to medical management we preferred side-to-side portal-systemic shunt.

Hepatic hemodynamics and portal venous anatomy can be assessed from preoperative visceral angiograms.[9,16,8] In our services we don't have this possibility in emergency, that's why this factor is theoretical; "time" factor frequently is against us.

The experience and competence of the surgeon is possibly the most important factor in selecting the surgical procedure for a patient who has bled from esophageal varices.[13] The surgeon must choose a "familiar" procedure. A "first technique" can be a "successful procedure" in elective conditions, but in emergency we must made a "classic operation", with minimal risks and very "fast".

The human factor, including here the patient and the surgeons and anesthesia team, must be correctly appreciated.

The "variceal bleeding "have the right to kill", but the "scientific hemorrhage does not".

In conclusions, we suggest to correlate all these factors, and to accept that in portal hypertension with variceal bleeding selection of the surgical procedure and an optimal timing of operation are the maturity characters of the surgical team.

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Surgical techniques used in the treatment of malignant tumors of the ampulla of Vater in Department of Surgery I Targu-Mures

A.Tudor[1], C. Copotoiu[1], Klara Branzaniuc[2], C. Nicolescu[2]

Introduction:Tumors of the papilla and ampulla of Vater are rare neoplasms which are usually detected at an early stage due to their symptoms. Three procedure are currently being used to treat such tumors: pancreatoduodenectomy(PD), transduodenal local excision(TDE) and endoscopic snare excision(ESE).
Pacients and Methods:Between 1995 and 2008, 47 pacients with adenocarcinoma of the papilla of Vater were operate on.The surgical procedures were: 23 pancreatoduodenectomy, 4 transduodenal local excision, 10 biliogigestive derivations and 10 exploratory laparotomies in metastatic cases.
Discussion and conclusion:The appropriate treatment for ampullary tumors is still under debate. Pancreatoduodenectomy (PD) is the procedure of choice in case of tumors papilla of Vater though still bearing 2% to 5% mortality and substantial morbidity of 30%-40%.
Key words: duodenopancreatectomy,malignant tumors of ampulla of Vater,trasduodenal local excision, endoscopic snare excision

The anatomy of the ampulla of Vater is very complex. It consists of three different epithelia (bile duct, pancreatic duct, and duodenum).

Adenomas are considered as precancerous lesions and they usually occur in the fifth-sixth decade of life. There is evidence supporting adenoma-carcinoma sequence of neoplastic lesions of ampulla of Vater.[14,24,26]

The frequency of malignant lesion in an adenoma of the papilla is about 26%.[3] In postmortem studies, carcinomas of papilla of Vater have been reported in 0.2% of cases.[16] Through increased use of endoscopy, ampullary tumors are more frequently recognized.

Differentiation of adenocarcinoma of the pancreas from ampullary tumors is also important considering better prognosis for long-term survival in the latter group. It is generally agreed that adenocarcinoma of papilla of Vater should be removed by partial pancreatoduodenectomy, whereas local ampullectomy with local lymphadenectomy should be reserved for pTis and PT1N0M0G1 or G2 tumors.[3]

Adenocarcinoma is the most common malignant tumor of the ampulla, but in general it is still rare. That is why these tumors are very difficult to study, and most reports considering ampullary tumors are of retrospective design.

PATIENTS AND METHODS

To analyze surgical techniques as well as risk factors, we have collected data retrospectively in a specially created database on 47 consecutive patients with adenocarcinoma of the papilla of Vater who had been operated at the Department of Surgery 1, Targu Mures.

Between January 1, 1995 and January 1, 2008, 47 patients with adenocarcinoma of the papilla of Vater were diagnosed and operated on.

Surgical techniques were various depending on tumoral stage, age, biological status of the patients. Risks factors, which affect postoperatory mortality and morbidity, were evaluated.

According to data from literature[12,17,20,21] mortality was more frequent at patients with severe jaundice, hepatic dysfunction, renal dysfunction and anemia.

Some authors claim that elderly patients and neoplastic disease are minor risk factors and it doesn't influence postoperatory mortality and morbidity.[8]

RESULTS

Twenty-three patients with malignant tumor of the papilla of Vater had undergone cephalic pancreatoduodenectomy. Preoperatory resectability was confirmed during surgery. The association between CAT-scan and laparoscopy is an efficient method for a better control of the resectability.

The procedure of choice in malignant and resectable tumors of the ampulla of Vater was defined: cholecystectomy, resection of the distal segment of CBP, resection of 2/3 of the gastric part,resection of the head of the pancreas , duodenectomy, resection of the initial segment of jejunum; lymph node dissection, including anterior and posterior lymph nodes of the pancreatic head, supraduodenal, common and proper hepatic artery, hepatoduodenal ligament, and lymph nodes to the right of the superior mesenteric artery.

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After resection time the reconstruction time follows; three anastomosis were performed: end-to-side hepaticojejunostomy, gastrojejunoanastomosis and pancreaticojejunostomy or pancreaticogastrostomy.

Pancreaticogastroanastomosis was performed in 17 cases, pancreaticojejunoanastomosis in 6 cases .

Transduodenal ampulectomy was indicated in 4 cases particulary in patients with first stage (intraampular stage). Several technical principles are important during local excision, not only to avoid postoperative complications but also to minimize the incidence of recurrence.

The most important goal to decrease the recurrence is to obtain an adequate margin, which is sometimes difficult in large tumors.

Tumor infiltrations of the bile and pancreatic ducts are equally important, with safe ductal resection limited to approximately 1cm. In papillary tumors which cannot be removed with adequate margins without compromising the ampullary orifice, it is necessary to excise the ampulla and formally reconstruct both bile and pancreatic duct orifices with fine interrupted sutures.

In 10 cases, due to the local extension of malignancy process, biliary-digestive derivation was performed. This procedures was: 6 cholecystogastroanastomosis, in two of this cases a gastroenteroanastomosis was also performed, because of the duodenal stenosis process and 4 choledocoduodenoanastomosis when this stricture doesn't exist. In 10 cases due to continuous evolution in peritoneal or hepatic areas, an exploratory laparotomy was considered to be the proper approach.

DISCUSSION

Surgical intervention, along with its risks and advantages, must be considered the appropriate solution in all these cases. The surgeon has then the opportunity to perform radical tumors resection and/or at least to obtain definite histological confirmation.

Pancreatoduodenectomy (PD)

This procedure now has low morbidity and a hospital mortality of 3%-9% in large series from experienced centers.[1,3,6,27] Due to favorable outcome of PD, some authors suggest radical resection as the optimal treatment , even for benign tumors.[4,9]

Long-term survival after PD for ampullary tumors has been reported by a number of authors, with conflicting results.Some series reported 5-year survival rates of up to 60%,[1,6,18,25] while others reported 5-year survival rates between 21% and 38%.[13,27]

Transduodenal local excision (TDE)

TDE of the papilla is indicated in papillitis, adenomyosis, or neurinoma of the papilla, and in small adenomas of the intraduodenal part of the papilla, particularly if there is a tubular adenoma without villous tissue components.The frequency of malignant lesion in villous adenoma of the papilla amounts to approximately 30%.[9] A villous adenoma of the ampulla of Vater is considered to be a premalignant lesion,and should be treated with a complete extirpation of the lesion by an ampullectomy.

Similar ampullectomy is performed in the case of an adenoma with a carcinoma in situ or a PT1N0MO\G1-G2 cancer of the ampulla(limited to the mucosa).[22,26]

Some authors propose local excision for small(<3 cm) pT1 and pT2 cancers in high- risks patients report a markedly decreased mortality and morbidity compared to PD.[2,3,7,10,15,18]

Endoscopic snare excision (ESE)

Endoscopic techniques for the management of tumors of the papilla of Vater continue to evolve. During the past decade, ESE of benign papillary adenomas has been proposed as an adequate method in comparison with PD or TDE, also forpatients at very high risk of extensive surgical procedures or those who refuse operation.[5,11,19,28,29]

Criteria for using ESE are tumor size less than 4 cm or no greater than half the circumference of the duodenum, absence of strictures and extension into pancreatic and biliary ducts, benign endoscopic formation with no ulcerative lesions, and benign histological results on at least six forceps biopsies.[5,11]

Concerning the potential malignancy of papillary adenomas, ESE of papillary tumors has the additional advantage of providing an adequate specimen for the final histopathologic evaluation, thus justifying an initial attempt of ESE, if technically feasible.

CONCLUSION

The appropriate treatment for ampullary tumors is still under debate.

Malignant tumors of the papilla of Vater should be treated by radical excision such as PD,because early tumor stages also demonstrate up to 30% spread into tumor lymph nodes.

Pancreatoduodenectomy, wich allows wide excision, is also justified in some benign neoplasms as it can be performed with low mortality and morbidity rates in experienced hands.

Therefore, the management of papillary tumors still remains controversial and interdisciplinary because of the complex anatomy of the ampullary region and the difficulty of accurately diagnosing and staging the tumor through imaging and endoscopy preoperatively.

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When do we determine the SOFA (Sequential Organ Failure Assessment) score for the assessment of organ failure in critical patients?

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It is obviously outlined that the occurrence of failure in organ systems is the major gravity indicator in the evolution of the critical patients.
Currently, the SOFA (Sequential Organ Failure Assessment) score is largely used for the assessment of MODS incidence and seriousness, considering its simplicity, accuracy and reliability.
The ideal time for the determination of the SOFA score in critical patients has not been clearly defined.
Within this observational study, we present our opinion about the initial time determination of the SOFA score with a view to establishing the MODS incidence, so that patients should be hospitalized in the intensive therapy department and that adequate managerial decisions should be made.
Key words: SOFA, MODS, critical patients, intensive care

The Multiple Organ Failure Syndrome-MODS is characterized by the failure of one or more organs in a critical patient, whose homeostasis cannot be maintained with no intervention (BONE, R, C, et all, 1992, cit. de 1, 2).

MODS appears in about one third of the critical patients hospitalized in the intensive therapy department, being the main cause for mortality. This is strictly related to the amount of affected organs / insufficient systems, 16-42% in patients with only one affected organ and 55-100% with those having three or more affected organs.

In time, several score systems have been imagined and used in order to assess the seriousness, evolution and prognosis of critical patients with organ failures.

Currently, the SOFA score (European Society of Intensive Care Medicine, Paris, 1994) is the most widely used one for the assessment of critical patients with MODS, being easily operational, accurate and reliable.[16,17]

The initial time for the determination of the SOFA score in critical patients with MODS is not clearly established.[14]

In this observational clinical study, we intend to present the data and our opinion concerning the initial determination of the SOFA score in critical patients, with a view to opting for the hospitalization in the intensive therapy department and in favour of a therapeutic behaviour.

MATERIAL AND METHOD

The SOFA score was determined in 15 critical patients hospitalized in the intensive therapy department in Targu Mureş and Braşov during the first hours of hospitalization (6-24 hours).

The SOFA score (Table I) was determined according to conventional calculations and the registration of the clinical/ paraclinical values respectively, the most abnormal of the 12 parameters of the 6 organs. This value is entered in the columns 1, 2, 3, 4, which is the partial score of the organ failure. The total SOFA score is calculated by adding up the worst values of the 6 organs with failures.

The patients whose SOFA score was negative were proposed for hospital leaving at the clinics of provenience or were attended and treated if vacant places available.

The SOFA score was determined daily in the case of patients who had at least one organ failure in order to observe the evolution (improvement or worsening), and the forecast under conditions of aggressive „support" therapy.

RESULTS

Out of the 15 critical patients for which the SOFA score was determined in the first hours, in 5 patients it was found negative, in 3 patients we established the failure of only one organ, and in 7 patients there was failure in three or more organs (Table II).

DISCUSSIONS AND CONCLUSIONS

This observational study is meant to establish the MODS with the help of the SOFA score, within the first 6-24 hours after hospitalization. The SOFA score is simple, accurate and reliable for the assessment of the seriousness of the patient's state, the forecast and the effect of the new therapies.[17]

Thus, based on the 12 parameters of the 6 organs / systems (breathing, renal, cardiovascular, liver,

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Table I. SOFA score (Sequential Organ Failure Assessment) European Society of Intensive Care Medicine, Paris, 1994

Organ/Sistem	0	1	2	3	4
Breathing PaO2/FiO2 (mmHg)	>400	<400	<300	<200*	<100*
Renal Creatinine (mg/dl) or urinary flow (ml/day)	<1,2	1,2-1,9	2,0-3,4	3,5-4,9 or <500ml/day	>5 or <200ml/day
Hepatic Bilirubin (mg/dl)	<1,2	1,2-1,9	2,0-5,9	6,0-11,9	>12,0
Cardiovascular (Hypotension)	no hypo tension	MAP<70mmHg	Dopamine <5 or Dobutamine (any dosis)**	Dopamine>5 or Adrenalin<0,1 or Noradrenalin<0,1**	Dopamine >15 or Adrenalin>0,1 or Noradrenalin>0,1**
Hematological Nr. booklets (x1000/mm³)	>150	<150	<100	<50	<20
Neurological GCS	15	13-14	10-12	6-9	<6

*with ventilator support
** adrenergic substances administrated at least one hour ahead (in mg/kg/min)
MAP = mean arterial pressure
GCS = Glasgow Coma Score

Table II. Representing the seriously sick hospitalized in the intensive therapy department, in which the SOFA score was determined in the first 6-24 hours

Total amount of patients		No organ failure		One organ failure		Three or more organ failures	
AN*	%	AN*	%	AN*	%	AN*	%
15	100	5	33,33	3	20	7	46,67

AN* = Absolute Number

hematological, and neurological), the SOFA score enables prompt assessment of the critical patients in MODS in an operative way by the intensive therapy physician with medium equipment.

Vincent J. L. and colab[17] uses the SOFA score for the occurrence and seriousness of organ failure in critical patients, which is an easy and efficient method. The regular repetition of the score allows for the monitoring of the patient's state and the evolution of the disease.

Arts D. C. T.and colab[8], in an observational study based on the collection of 15 critical patients from 20 ICUs, determine the SOFA score in order to quantify the seriousness of the disease based on organ dysfunction, at the same time noticing the simplicity, accuracy and reliability of the score.

Ferreira F. L. and colab[4], assessing the organ failure in critical patients with the SOFA score method observe that irrespective of the initial score, its increase in the first 48 hours means a mortality rate of at least 50%.

Forte D. N. and colab[5] establish that the SOFA score determined on the first day in patients with MODS has the highest accuracy level to foresee the mortality in ICU.

Janssens V. and colab[12] show that the SOFA score offers the clinical doctor important information about the degree and progress of the organ failure, in medical patients identifying the patients with high risk in the intensive therapy department.

Sogayar A.C.B. and colab[14] assert that the ideal SOFA score assessment time "isn't clearly established", coming up with the hypothesis that it has to be applied after the initial 24-hour treatment, being more valid than in ICU for the result forecast.

Gurman G[6] states that none of the initiators of the patients' state quantifying methods (APACHE II, SAPS, etc.) has proposed that these methods be used for selection regarding the admission in the intensive therapy department. The author also shows that the scores have rapidly become objective instruments in the clinical doctor's hand to help in taking decisions regarding the selection of patients for the intensive therapy department.

The correct selection of the patients for the admission in the intensive therapy department has a twofold scope[6]:

-To prevent the admission of patients who are not in imminent vital danger, respectively the limitation of those outside the therapeutical resources.

-To keep the resources – that are anyway limited – for the patients with highest survival chance.

Traditionally, the admission of critical patients in the intensive therapy units occurs at the proposal of the curing doctors based on the „clinical judgment"[6] and possibly at the family's pressure, without routinely appealing to the seriousness scores.

In our study we could show that out of the 15 critical patients brought into the intensive therapy section in which we determined the SOFA score, 5 did not present organ failure, being treated with conventional methods, whereas 10 patients displayed failures in one or several organs. In these ones we repeatedly determined the SOFA score with the purpose of establishing the evolution, forecast and the prompt recommendation of the „support" therapies.

Thus, we conclude that, in our opinion, the SOFA score assessed in the initial hospitalization hours can settle the beginning of MODS, indicating the admission in the intensive therapy with the change of conventional managerial behaviour into a more aggressive „support" behaviour with changed medical, ethical and financial consequences.

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Abdominal perfusion pressure as an outcome prognosis factor in critically ill patients with abdominal compartment syndrome: a metaanalysis

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Numerous injuries that lead to patient's admission in the ICU will develop in time abdominal compartment syndrome (elevated IAP). Abdominal perfusion pressure combines the absolute IAP and the physiologic parameter of mean arterial pressure (MAP) to better asses abdominal end organ perfusion. It could be superior to IAP, pH, base deficit and arterial lactate in predicting patient outcomes.

Pubmed was searched using 5 key words. Out of the 368 studies (18 related to outcome), 4 were included. They recruited 1066 patients. Data were analyzed in MIX. APP appeared as a good outcome predictor, especially for values above 60 mmHg. Abdominal perfusion pressure may become the most useful parameter to guide clinicians in their resuscitation and management of patients suffering from ACS.

Key words: abdominal perfusion pressure, outcome, critically ill patient

Abdominal compartment syndrome is a clinical disease spectrum that results from elevated intra-abdominal pressure (IAP) due to tissue edema or free fluid collecting in the abdominal cavity. The primary pathophysiologic event leading to intra-abdominal hypertension and the abdominal compartment syndrome (IAH/ACS) is interstitial edema in the bowel and mesentery due to capillary endothelial damage. It occurs due to ischemia from the original physiologic insult (sepsis, hemorrhage, etc) or/and due to secondary damage from the pro-inflammatory cytokines released in response to this insult. Occult hypovolemia was described.

Elevation of IAP has serious impact on organ perfusion throughout the body. It leads to reduced cardiac output, increased pulmonary and systemic vascular resistance.[1] Traditional pressure based measurements of vascular volume status such as CVP and PCWP are erroneously elevated and do not reflect the patients volume status. IAH prevalence ranges from 4% to 80% (when it comes to purely surgical patients) with a mean of 50% for either medical or surgical ICUs.

Although intra-abdominal pressure clearly correlates with patient outcome[1], the IAP at which an individual patient develops problems is highly variable and dependent upon a numerous factors including patient co morbidities and mean arterial blood pressure.

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The amount of blood flow (oxygen) delivered to intra-abdominal tissue is the result of the difference between the pressure resisting flow (intra-abdominal pressure: IAP) and the pressure delivering that blood (mean arterial pressure: MAP). It is known as abdominal perfusion pressure.

We intended to assess the impact that APP has on outcome thus supporting the idea that early goal directed therapy to improve tissue perfusion and oxygen delivery is essential to ensure optimal survival and reduced morbidity in critically ill patients.

MATERIAL AND METHOD

Pubmed was searched using 5 key words. The time span was 1990-2009. Out of the 368 studies (18 dealing with outcome), 4 were included (due to heterogeneity).[2,4,6,7]

The entry criteria used were: Diagnosis of Abdominal Compartment Syndrome established as IAP>20mmHg and/or IAH>12mmHg[3] correlated to the outcome, thus meaning survival at discharge or at 90 days. Case reports with less than 4 patients were discarded. Thus, only 1066 patients could be included in the study. No other studies correlated survival to ABS or IAH.

Data were analyzed in MIX 1.7 (Meta analysis made easy).

RESULTS AND DISCUSSIONS

APP was assessed as an outcome predictor (survival) in patients with IAP and ACS compared to

MAP and SAPS. Table I depicts sensibility and specificity for predicting patient survival (Cheatham)[2] and mortality (Malbrain – single center pilot study and multicenter study).[5]

The cut-off points of APP at 60 mmHg, MAP at 70 mmHg and IAP at 9-12 mmHg have the best sensitivity and specificity for patient outcome. The differences between the Cheatham study and the others may be related to disparity in IAH severity and patient population between the trials.[2,5] The highlighted values are included in the WSACS's recommendations with an IC EBM level.

Table II depicts AUROC curves predicting hospital survival while table III reflects ICU mortality.

Stated otherwise, a low IAP would be as good a predictor for survival as a high APP.

Table IV summarizes APP and MAP in survivors and non-survivors with IAH versus the general population.

Persistence of IAH and failure to maintain APP at or above 60 mmHg by day 3 was found to discriminate between survivors and nonsurvivors. The therapeutic threshold above witch raising MAP to achieve a particular APP becomes futile or even detrimental remains unknown. Indiscriminative fluid administration places the patient at risk for secondary ACS. Target APP should be achieved through a balance of judicious fluid resuscitation and vasoactive medication.

One of the WSACS (World Society of the Abdominal Compartment Syndrome) active clinical trials submitted march 2008 addresses this issue.[9] It is a

Table I. Sensibility and specificity for predicting patient survival (Cheatham[2]) and mortality (Malbrain – single center pilot study and multicenter study

Variable	Sensitivity			Specificity		
	Cheatham	Pilot	CIAH	Cheatham	Pilot	CIAH
APP						
40	0.53	0.14	0.14	0.78	0.98	0.95
50	0.76	0.39	0.53	0.55	0.91	0.85
60	0.92	0.55	0.79	0.25	0.76	0.62
MAP						
60	0.23	0.34	0.40	0.87	0.87	0.87
70	0.57	0.58	0.69	0.60	0.64	0.61
80	0.83	0.74	0.85	0.21	0.42	0.41
IAP						
9		0.65	0.91		0.72	0.26
12		0.44	0.75		0.93	0.59
15	0	0.23	0.47	0.96	0.97	0.75
20	0.08	0.06	0.17	0.84	0.99	0.92

Table II. AUROC curves predicting hospital survival

Variable	Cheatham[2]	Pilot	CIAH	Reintam[7]
APP	0.730	0.690	0.731	0.461
MAP	0.620	0.641	0.708	NA
IAP	0.290	0.241	0.322	0.470

NA not accounted

Table III. AUROC curves predicting ICU mortality

Variable	Cheatham[2]	Pilot	CIAH	Reintam[7]
APP	0.270	0.310	0.279	0.539
MAP	0.380	0.358	0.292	NA
IAP	0.709	0.758	0.678	0.530
SAPS II	NA	0.883	0.718	NA
APACHE II	NA	NA	NA	0.876
SOFA	NA	NA	NA	0.871
Lactate	NA	NA	NA	0.771

NA not accounted

Table IV. APP and MAP in survivors and non-survivors with IAH vs. general population

Study	Variable	IAH		General population	
		Survivors	Non-survivors	Survivors	Non-survivors
Pilot	APP	57.7±30.1	52.6±19.7	75.7±22.6*	60.8±23.1*
	MAP	72.4±29.6	68.8±18.9	82.5±22.2*	72.2±21.5*
	IAP	14.7±2.6	16.2±3.9	6.9±3.6*	11.4±5.3*
CIAH	APP	64.9±22.6*	51.8±13.5*	69±23*	54.2±15.5*
	MAP	80.8±22.4*	67.9±13.5*	81.3±22.5*	68.3±14.9*
	IAP	17.1±4.9	16.6±3.7	11.8±5*	15.1±5.6*

* statistically significant (p<0.001)

prospective randomized trial of APP as a resuscitation endpoint. Its aim is to determine whether maintenance of APP 60 mmHg improves survival in patients with either moderate intra-abdominal hypertension (IAH) or ACS (abdominal compartment syndrome) when compared to a traditional MAP (mean arterial pressure) resuscitation endpoint of 65 mmHg.

It could be argued that IAH may be just one of the symptoms reflecting the severity of critical illness, lacking a specific value in clinical practice (IAP on intensive care admission has no prognostic value). However, the development of IAH was identified as an independent risk factor for mortality. The only study[7] that discriminates between primary and secondary IAH confirms the fact that the latter is less frequent, has a different time course and worse outcome than the primary IAH (due to the multifactorial etiologies and no specific treatment – no use for the decompressive laparotomy).

There is more to a functional parameter that meets the eye.

CONCLUSIONS

Although APP appears as a promising outcome predictor, further studies are needed to evaluate its use to guide ACS management as an early goal directed therapy target to be achieved.

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Survival rates after cardio-pulmonary resuscitation in the Emergency Department, Targu Mures

V.T.Ghitescu[1], Oana Diana Dimulescu[2], G. Gyorfi[2], Ioana Ghitescu[3], C. Boeriu[4]

Survival rate following cardiac arrest remains very low, despite the attempts made to identify the determinants of outcome. Objective: To determine the impact of the main diagnosis on survival following cardio-pulmonary resuscitation (CPR). Methods: retrospective study, between 01.01- 01.06.2008 in Targu Mures Emergency Department, on 180 patients, mean age of 65.11±14.82 years, who were submitted to standard CPR followed by return of spontaneous circulation (ROSC). We identified three groups according to the DRG classification of the diagnosis: A (n=62, cardiology); B (n=42, neurology); and C (n=76, other). We analyzed the time between ROSC and death/ transfer and we assessed the 300 hours survival after ROSC. We compared the demography between groups (age: ANOVA; sex: $\div^2$). We made a Kaplan-Meyer estimation of survival rates in the three groups using log-rank test on GraphPad Prism 5.0. software. Results: there were no differences between groups for age (p=0.0992), or sex (p=0.1450). Mortality at 300 hours after ROSC was 40.32% (A), 26.19% (B), 44.73% (C). We found no differences between the three Kaplan-Meyer survival curves, p= 0.1247. Conclusions: Survival at 300 hours after ROSC is not influenced by the diagnosis that led to cardio respiratory arrest.

***Key words**: cardio respiratory arrest, outcome, survival*

Since the introduction of the cardiopulmonary resuscitation (CPR) with mouth-to-mouth respiration and closed chest compression by Safar and Kouwenhoven in the early '60s[2,6], efforts in the resuscitation studies have been made to analyze the items that determine the return on spontaneous circulation (ROSC), with less attention paid on overall survival rate and long term survival. It was considered that improvement in outcome of cardiac arrest patients would lie in shortening the period of circulatory stop. In recent years several studies have demonstrated an increased survival for the cardiac arrest patients admitted to the intensive care units, but still having an extremely poor outcome, especially in the terms of neurological recovery.[3,7] The prolonged period of systemic ischemia followed by reperfusion after ROSC is the main determinant of the post-cardiac arrest syndrome.[4] However, it is not known whether the main diagnosis that led to a successfully resuscitated cardiac arrest has an important impact on short / medium term outcome.

Objectives. To determine whether there are statistically significant differences between short term survival (300 hours after ROSC) for patients with different types of diagnosis that led to the cardio-respiratory arrest event.

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MATERIALS AND METHODS

We conducted a retroactive study with the approval of the medical committee of the University Emergency County Hospital, Targu Mures, (file no. 840/20.01.2009), in the Emergency Department, during 01.01-01.06.2008. We analyzed the medical recordings of the patients resuscitated in this department and included in our study only the patients aged over 18 with return of spontaneous circulation (ROSC) after standard cardio-pulmonary resuscitation (CPR) performed according to 2005 guidelines.[1] We finally included in our study 180 patients, mean age 65.11±14.82 years, 118 male and 62 female, divided in three groups, according to the diagnosis completed at the conclusion of the patients' recordings and the DRG classification. We considered as most important groups of causes of cardiac arrest, according to their incidence, the heart-related diseases (cardiology), the neurological diseases and a third group of miscellaneous diseases (hematology, pulmonary, oncology, traumatology and poisoning). According to this division we named: group A, patients with cardiac diseases that determined the cardiac arrest, with n=62 patients mean age 65.77±13.49 years, 35 male, 27 female; group B, patients with neurological diseases that caused cardiac arrest, with n=42 patients, mean age 63.88±18.83 years, 28 male and 14 female; group C, patients with other main diagnosis with n=76, mean age 65.25±13.46 years, 55 male and 21 female. We compared age between groups using ANOVA test and gender distribution using $\div^2$ test. We calculated the time between ROSC and death/ transfer/ discharge and recorded survival at 300 hours as follows: the patients who died before the completion of this interval were

noted with 1; the patients who were transferred before 300 hours after ROSC were noted with 0 and calculated as censored values; the patients who survived after 300 hours were considered survivors and noted with 0. The time of ROSC and the time of transfer were considered the time registered in the emergency department file by the medical staff that performed CPR, and further referred the patient; the time of death was considered the moment of the death diagnosis as registered in the hospital's files. The time intervals and the code 0/1 were registered in a Microsoft Excel worksheet, divided into three according to the above mentioned groups A, B, C , transferred and analyzed in a Graph Pad file using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. We used this software to make the descriptive analysis of the study population, the ANOVA and χ^2 test for comparing the demographic characteristics of the three groups, the Kaplan-Meyer curve for assessing the risk of death for each group and the log-rank test for the statistical comparison of the three Kaplan-Meyer curves. All results were considered statistical significant for a p value< 0.05.

RESULTS

We found no differences between the three groups for age and sex distribution (Table I). Mortality at 300 hours after ROSC was (Table I): 25 patients / 62 in group A (40.32%), 11 patients/ 42 in group B (26.19%) and 34 patients/ 76 in group C (44.73%).

When calculating the Kaplan-Meyer curves for estimation of survival rates for each group (Figure 1), we obtained two almost superposed curves for groups A and C, and a higher curve (lower risk of death) for group B.

Median survival time in group A was 270 hours, in group B was undefined (more than 50% of subjects were survivors/ censored at 300 hours), and 199 hours for group C. Comparing the three survival curves with log-rank test, although group B seems to have a lower mortality risk at 300 hours, we found no statistically significant difference between the survival proportions, p=0.1247 (degrees of freedom =2). When comparing the same curves two by two, we obtained the same results, as follows: p= 0.1634 (A versus B, degrees of freedom 1), p= 0.3651 (A versus C, degrees of freedom 1), and p=0.0589 (B versus C, degrees of freedom 1), so there is no statistically significant difference for survival at 300 hours between the three diagnosis groups.

DISCUSSION

Our study, although retrospective, highlights a very important part of the medical, ethical and not ultimately financial aspect of the resuscitation medicine. Although several factors have been shown to influence the outcome after CPR for cardiac arrest in terms of ROSC and short term survival, prognostication of futility remains controversial.[5] At this moment it can be stated that prognosis cannot be based on the circumstances surrounding cardiac arrest and CPR, and

Table I. Characteristics of patients and mortality at 300 hours after ROSC

Characteristics	GROUP A (n=62 cardiology)	GROUP B (n=42 neurology)	GROUP C (n=76 other)	*p* value
DEMOGRAPHY				
Mean (SD) age (years)	66.5±13.49	63.88±18.83	65.25±13.46	0.9266 (ANOVA)
Min/max age (years)	23/91	18/87	23/87	
Sex (M/F)	35/27	28/14	55/21	0.1450 (χ^2)
MORTALITY	25p/62=40.32%	11p/42= 26.19%	34p/76=44.73%	

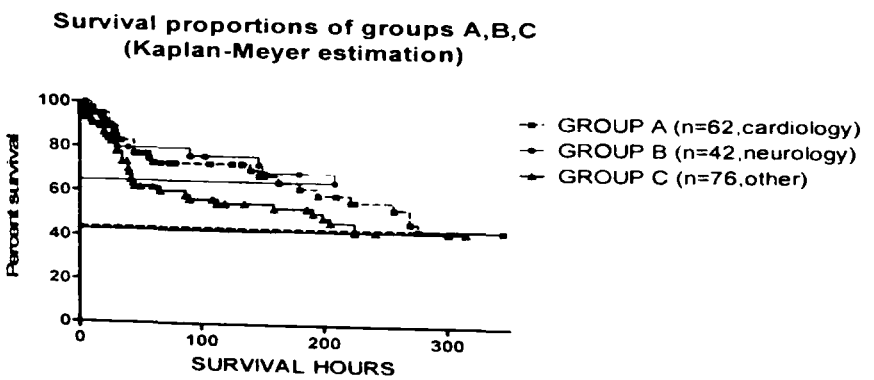

Figure 1. Kaplan-Meyer estimation of survival rates in groups A, B, C.

the reliability of all prognosticating tools is dependent on when they are applied after cardiac arrest.[5] That is why our findings are important in our opinion, because they are highlighting the fact that a short/medium time survival after cardiac arrest is not influenced by the primary diagnosis that led to the cardiac arrest event; expressed in another way, analyzing cardiac and neurological causes of cardiac arrest, none of these types of diagnosis represents a favorable survival factor at 300 hours (more than 12 days). We believe that the real outcome (at 12 days) of the successfully resuscitated cardiac arrest, with ROSC is superposed on the prognosis of the post-resuscitation syndrome, as a form of systemic inflammatory response syndrome, preceding the development of multiple-organ dysfunction syndrome. These findings suggest that prolonged multiple organ support and time-critical intensive therapies provided in the intensive care units may increase the chances of survival after ROSC following CPR, but also that these efforts must be made irrespective of the primary diagnosis that led to the cardiac arrest. It is well-known that treatment limitations within the first 24 hours and establishing DNR orders shortly after cardiac arrest and ROSC leads to a decreased chance of survival.[3,8] We believe also that declaring futility, judged in terms of short/medium time survival, and treatment limitations should be considered without taking into account the primary heart-related or neurology-related diagnosis.

CONCLUSION

Survival at 300 hours (more than 12 days) after ROSC following CPR is not influenced by the diagnosis that led to cardio-respiratory arrest.

This outcome is probably related to the severity of the post-resuscitation syndrome. Outcome for these patients is subject for important discussions linked to the quality of the survival as defined by the myocardial dysfunction, and especially, by the recovery of the neurologic function. For successfully resuscitated patients the long-term neurologically intact survival is the real and the most important post-resuscitation outcome, and efforts should be made in identifying the prognostic items linked to this condition.

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Adequate perioperative analgesia reduce postoperative morphine requirement in major abdominal surgery

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The management of pain remains a major concern in the perioperative period, not only because pain affects surgical outcome, but postoperative pain also may result in the development of chronic pain even after surgery. We performed a prospective, randomized, study on 16 women scheduled for major abdominal tumour surgery to test the hypothesis that additional preoperative epidural infusion of ropivacaine can reduce the postoperative pain perception and requirement of analgesics in comparison with only systemic intraoperative analgesia. Visual analogue scale (VAS) pain scores, evaluated at rest and during coughing, the total administered dose of morphine were evaluated at admission to the ICU and then at 2, 4, 6, 12, 24 and 48 hr after surgery. The results of the present study show that blocking or attenuating noxious inputs before incision, (preemptive analgesia) with epidural ropivacaine, reduced the intraoperative requirements of iv fentanyl and postoperative morphine consumption, as a rescue analgesic. Although the magnitude of this effect was modest, pre-surgical administration of epidural ropivacaine reduced pain on movement, in the early postoperative period after major abdominal surgery.

***Key words**: preemptive analgesia, ropivacaine, visual analogue scale*

The management of pain still remains a major concern in the perioperative period, not only because pain affects surgical outcome, but postoperative pain also may result in the development of chronic pain even after surgery.[2,7]

Pain associated with tissue damage, once initiated, will launch a cascade of alterations in the somatosensory system and will increase the responsiveness of both peripheral and central pain pathways. It is preferable to prevent the neurophysiological and biochemical consequences of a noxious input to the CNS rather than to begin treatment after these consequences are already established.[7]

Preemptive analgesia (preemptive antinociception) it is a term that refers to the concept that by blocking neuronal pathways before or during injury, we can reduce or eliminate the hyperexcitability of these pathways and pain memory during recovery.[3,6] In a recently published review Moiniche et al. pointed out that preemptive epidural treatment might have an improved capacity to reduce nociceptive input caused not only by incision and ongoing surgery but also by postsurgical inflammation.[4,5]

We performed a prospective, randomized, study on 16 women scheduled for major abdominal tumour surgery to test the hypothesis that additional preoperative epidural infusion of ropivacaine can reduce the postoperative pain perception and requirement of analgesics in comparison with only systemic intraoperative analgesia.

MATERIAL AND METHOD

After institutional ethics committee approval and written, informed consent,16 women, scheduled for major abdominal surgery, (gynecological tumour surgery), planned to last at least 1 h and requiring morphine for postoperative analgesia, were included. Patients were admitted in C.F.R. Hospital of Craiova between 1st January 2008 and 31st June 2008.

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Exclusion criteria were ASA more than III, age younger than 18 years or older than 80 years, allergy to local anesthetics or opioids, current opioid or any kind of analgesic medication use, active infectious process, neurological disorders, abnormal coagulation tests, renal or hepatic failure, lack of cooperation. We also excluded patients with pre-existing pain with a visual analogue scale (VAS) score more than 10 mm.

On the day before surgery, patients received instructions on how to measure pain with a visual analog scale (VAS) that consisted of an unmarked 100-mm line, with 0 mm representing no pain and 100 mm representing the worst pain imaginable.

On the day of surgery patients were premedicated orally with 0.1 mg kg^{-1} midazolam 60 min before arriving in the anesthesia room where they were monitored by an electrocardiogram, noninvasive measurement of blood pressure and pulse oximetry. A venous line was inserted and infusion of 500 ml Ringer's solution was started. With the patient in the sitting position an epidural catheter was inserted. A deep skin infiltration with 3–5 mL lidocaine 1% was performed for local anesthesia, before the 18-G Tuohy needle was inserted at the T7/8 using the loss of resistance technique. A test dose of 4 mL of lidocaine 2% with 1/100,000 of epinephrine was injected after negative aspiration.

Prior to induction of general anesthesia, the patients were randomly allocated into two equal groups, to receive a epidural bolus of 10 mL NaCl 0.9% in Group I or 10 mL bolus of ropivacaine 7.5 mg/mL in Group II.

General anesthesia was induced with propofol (2 mg/kg), fentanyl (0.2 µg/kg), and atracurium (0.5 mg/kg) and maintained with sevoflurane in 50% oxygen/air mixture. Patients received repetitive bolus of fentanyl for analgesia depending on predefined clinical criteria tachycardia > 90 beats/min and mean arterial pressure (MAP) > 90 mmHg. The lungs were mechanically ventilated. At the end of surgery, anesthesia was discontinued, the fentanyl was antagonized by nalorphine 0,2 mg/kg and residual neuromuscular blockade was antagonized by neostigmine 40 g/kg and atropine 20 mg/kg. The trachea was extubated when the patient became fully awake.

After completion of surgery (end of skin closure) patients of both groups received a continuous epidural infusion of of 14 ml/hr of ropivacaine 2 mg/mL, for at least 48 hr.

The patients were transferred to the intensive care unit (ICU) for close monitoring over the next 48 hr. During the entire postoperative observation period patients were allowed to receive morphine 0.15 mg/kg sc as a rescue analgesic if pain at rest was > 40 mm on the VAS.

VAS pain scores, the total administered dose of morphine were evaluated at admission to the ICU and then at 2, 4, 6, 12, 24 and 48 hr after surgery. Pain scores using the visual analogue scale (VAS) (0–100) were evaluated at rest and during coughing.

Continuous variables are presented as mean ± SD, and categorical variables are represented as a number. Analysis of variance for repeated measures for two criteria or the Student's t-test were used to compare continuous variables when appropriate. Categorical data were analyzed using Fisher's exact test. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSIONS

All patients underwent standardized surgery (longitudinal abdominal incision from the pubic symphysis to the sternum, radical resection of the ovaries, uterus and inguinal lymph nodes). Patient's demographic characteristics such as age, height, weight and ASA status, were comparable among the two groups. One patient was excluded from statistical analysis, due to accidental removal of the epidural catheter.

The cumulative intraoperative dose of fentanyl was significantly higher in Group I when compared with Group II.

Upper and lower levels of sensory block did not differ between groups during the entire observation period.

VAS pain scores at rest were equivalent among the groups, but patients in Group I experienced more pain during coughing and mobilization measurement at 6 and 8 hr after ICU admission.

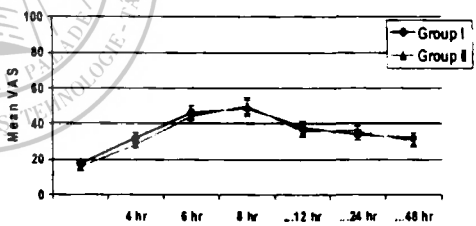

Figure 1. VAS values for pain assessed by patients at rest

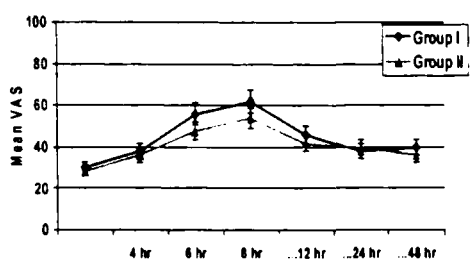

Figure 2. VAS values for pain assessed by during mobilization

We found a significantly increased analgesic effect between 8 and 12 hr after surgery in the Group II, cumulative morphine consumption being significantly lower ($P < 0.01$).

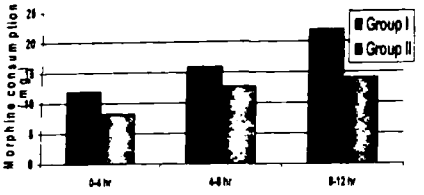

Figure 3. Cumulative morphine consumption in 4 hr intervals (first 12 hr)ups

The time to the first morphine rescue bolus use was shorter in Group I ($P < 0.01$) then in Group II. Cumulative morphine consumption (mg) at 12 rh was significantly ($p < 0.1$) lower in Group II (14.7±6) vs Group I (23.7±4), but total dose of morphine administered at 48 hr were not significantly different between groups.

Table I. Comparison of morphine administration in the two study gro

	Group I	Group II	P-value
Number of rescue bolus	4 (3–5)	1.5 (0–4)	<0.01
Time to first rescue dose (min)	54+/-12	82+/-6	<0.01
Total 12 hr dose (mg)	23.7 (±4)	14.7 (±6)	<0,1
Total 24 h dose (mg)	39.7 (±7.5)	36.5 (±1.2)	-

The side effects and complications associated with epidural anesthesia were minimum.

Relevant motor block (Bromage scale > 1, unable to rise knees against gravity) was observed in one patient of both groups. In GII one patient showed motor dysfunction (Bromage Grade 2) at 48 hr after the start of epidural infusion which was not detectable thereafter. No radicular irritation were seen up to the twenty postoperative day in any of the patients.

The results of the present study show that blocking or attenuating noxious inputs before incision, with epidural ropivacaine, reduced the intraoperative requirements of iv fentanyl and postoperative morphine consumption, as a rescue analgesic. The higher dosage of intraoperative opioids in Group I probably compensated the lack of epidural blockade, in order to provide analgesia comparable to the preemptive technique in Group II.

Although the magnitude of this effect was modest, pre-surgical administration of epidural ropivacaine reduced pain on movement, in the early postoperative period and it was associated with a short-term significant reduction in morphine consumption between 8 and 12 hr after surgery during the 48 hr study period.

We also show that preemptive epidural analgesia was clinically useful in prolonging the time to first analgesic request.

Our observation supports the findings of previous studies, that preoperative epidural analgesic treatment is useful in managing acute postoperative pain.[1,10] Subramaniam and colleagues reported improvement of postoperative pain after pre-surgical analgesia with epidural morphine and bupivacaine.[8]

The effect of intraoperative administration of ropivacaine on the development of chronic pain was not assessed in our study.

In the absence of the neuroprotective effect of the epidural agents, central sensitization was induced by surgery and maintained by postoperative inflammatory inputs from the wound. The neurophysiology underlying these effects has been well described and involves an N-methyl-D-aspartate–mediated increase in dorsal horn neuron activity.[2,4] Preoperative blockade followed by prolonged blockade of noxious inputs in the postoperative period may prove to be the most effective way of managing postoperative pain since the contribution to central sensitization of both noxious intraoperative inputs and postoperative inflammatory inputs may be prevented.[9]

CONCLUSIONS

In conclusion, our observation show that preemptive epidural analgesia with ropivacaine improve analgesic consumption, time to first rescue analgesia, but it doesn't reduce significantly postoperative pain scores.

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Primary cerebral hydatid cyst and sudden death: case report

L.Hecser, D.Biriş, Katalin Palfi Siklodi

Hydatid cyst is most frequently located in the liver (50-55%) and lungs (15-35%). Primary intracranial cysts are very rare (1.7%) and 75% of patients with intracranial cysts are children. The disease is most frequently seen in the area perfused by the middle cerebral artery; most cerebral hydatid cysts are supratentorial. The growth of the cyst occurs at various rates. The cerebral intraventricular manifestations of hydatid disease, which are very rare are characterized by undefined mechanisms of intraventricular involvement.
Authors present a case of cerebral hydatid cyst in a 49-year-old man with sudden death.
Key words: cerebral hydatid cyst, sudden death

Hydatid cyst is still an important disease.[2,15,27,32] *Echinococcus granulosus* is a parasite living in the intestines of animals like dogs wolves, and coyotes, whereas the larval cause hydatid cyst disease in humans, cattle, and sheep.[33] Hydatid cyst is mainly encountered in countries where cattle and sheep raising are important[1,19,29] such as Southern Europe, South America, Africa, Turkey, Australia, New Zealand, and India. Hydatid cyst is most frequently located in the liver (55-70%) and lungs (15-35%). In Croatia, it is most common in Dalmatia and in the neighboring countries of Bosnia and Herzegovina, Montenegro and Macedonia.[2] Primary intracerebral cysts are very rare (1.7%) and 75% of patients with intracranial cyst are children.[6,7,15,19]
The disease in most frequently seen in the area perfused by the middle cerebral artery.[29,31] The literature on intraventricular hydatid cysts consist of about 30 cases in which are described in large series[1,7,22] incidence are 1.3-16.6%.

We report a case of intracerebral hydatid cyst in a man with sudden death.

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Case report

A 49-year-old man in antecedents have nausea, vomiting, and headache. The person was found dead. The forensic autopsy (nr.443/2008 IML Tg.Mureş) to be found cerebral cyst (3x2x2cm) in lateral parts of the thalamus, and cardiohypertrophy. The cyst were without rupture

Figure 1. Cerebral hydatid cyst (aut. nr.443/2008)

The cyst was attached to the wale of the right lateral ventricle. Pathological examination confirmed hydatid cyst, and revealed the germinative membrane of the hydatid cyst

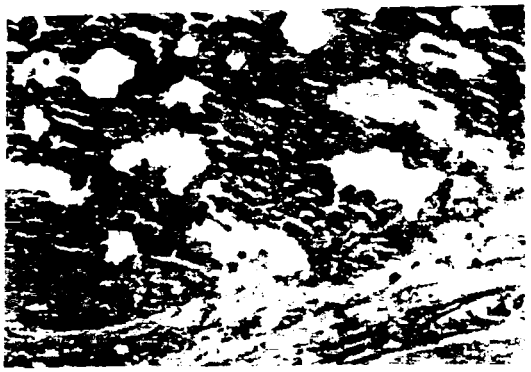

Figure 2. Cerebral hydatid cyst (well – content; Hpt.nr.20210/2008, col. HE x 100)

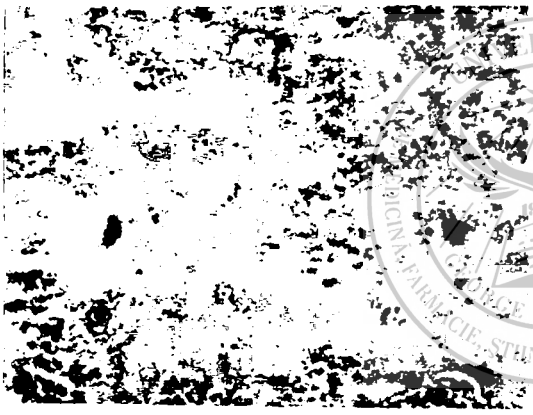

Figure 3. Cerebral hydatid cyst (content; Hpt.nr.20210/ 2008, col. HE x40)

In the present case, no hydatid cyst could be identified in organs other the brain so the brain was accepted as the primary focus.

DISCUSSIONS

Echinococcus granulosus parasitized dogs, jackals, and wolves. Human infections develop upon ingestion of the eggs excreted in animal feces. Any organ can be involved by the cyst formation, most frequently the liver (50-70%) and lungs (20-30%); however, other organs may also involved, e.g., the eye, heart, brain or muscle (less than 10%).[17]

Primary intracranial hydatid cysts are generally solitary, whereas secondary cysts are multiple. Secondary hydatid cyst is formed either by spontaneous or surgical regurgitation of a cyst localized in the left ventricle or in large vessels or traumatic or surgical rupture of the primary hydatid cyst so tend to be multiple in the brain.[12,25,33]

Headache and vomiting due to intracranial pressure increase, are the most frequent symptoms of brain hydatid cyst.[33] Neurological examination many reveal deteriorating consciousness, loss of strength, papilledeme, and pathological reflexes. Cranial CT shows hydatid cyst as a cystic, hypointensive, spherical lesion without peripheral edema.[9,14,28] If the cyst is infected, the contrast medium is retained as a peripheral ring and peripheral edema is present.[4,13,19] Hydatid cyst may be confused with cystic astrocytoma and brain abscess on CT, but can be differentiated by the absence of peripheral edema and mural nodule, and failure to retain contrast medium with latter disease.[16]

Because the cyst grows slowly, the disease may be asymptomatic for a long period of time.[22] The sign and symptoms of this parasitic infection are related to the site and size of the lesion. Discharge of the cyst content or cyst rupture can induce an allergic reaction to echinococcal antigen, including anaphylactic shock.[2,10,27] The growth of the cyst occurs at various rates. Ersahin et al.[11] reported that the growth rate of hydatid lesions was about 1 cm/month over a 6-month period in their study subjects, but Altinors et al.[3] and Sierra et al.[29] observed a progression in cyst growth of 7 mm/month and 5 cm/year, respectively.

Headache is usually the earliest symptom. The Weinberg and Cason serologic tests are of little practical value in confirming the diagnosis of cerebral echinococcall disease.[18,23,32] Most cerebral hydatid cysts are supratentorial, they involve the middle cerebral artery, presumably due to the cysts are sometimes localized in the fronto-parieto-temporal subcortical region.[9,14,17,18] There are 2 histogenetic types of cerebral hydatid cysts: primary and secondary.[15,33] The cerebral intraventricular manifestations of hydatid disease, which are very rare, are characterized by undefined mechanisms of intraventricular involvement. MRI studies, which also reveal the cyst capsule, can be used to assess the number of intracerebral lesions and to define the anatomic relationship of those lesions of the adjacent structures – details that cannot be easily seen on a CT scan.[3,5,7,14] Nevertheless, sometimes even with advanced imaging techniques such as CT and MRI studies, the diagnosis remains problematic.[3]

Hydatid cyst is often placed on cerebral hemispheres and is rarely placed on thalamic region. Kurtsoy et al.[20] reported thalamic hydatid cyst. Parietal region is reported to be the most common location of cerebral hydatid cyst.[7,10,11,20,27,26,30]

Hydatid disease is generally a severe disease, with over 90% mortality in untreated patients. Cerebral hydatid cyst should be treated both surgically and medically.[6,8,23] Medical therapy requires administration of 15 mg/kg albendazole for 3 month.[5,18] Albendazole has been used successfully for hydatid cysts in the liver, lung, and recently in the brain. Surgery is the standard and most effective treatment for intracranial hydatid cysts. The goals of surgery are the removal of the entire

intact cyst within rupture and the prevention of parasitic spread and additional neurologic deficits.[3,7,12,17] The removal of solitary cerebral hydatid cysts can be easily achieved without additional damage by using Dowling's technique.[3,4,7,11,12] However, the excision of large cysts may cause of sudden decrease in pressure followed by cerebral edema, hyperpyrexia, cardiorespiratory failure, subdural collection and the development of a porencephalic cyst. Rupture is associated with the well-recognized problems of anaphylaxis, meningitis or local recurrence from spillage of the cyst contents.[3,7,12,18,21,24]

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Langerhans cell histiocytosis: diagnostic difficulties and therapeutic approach. Case presentation.

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Langerhans cell histiocytosis is a group of idiopathic disorders characterized by the proliferation of cells of the mononuclear phagocyte system and the dendritic cell system. From the clinical point of view there are 3 forms of histiocytosis encountered in child pathology: acute, disseminated called Abt-Letterer-Siwe (it usually affects children under 2 years of age), eosinophilic granuloma and the intermediate clinical form called Hand-Schüller-Christian disease. We present the case of 15 years old boy with a symptoms combined: some that belong to one of the clinical forms and some of the other. Based on the clinical exam and the biological and functional data the positive diagnosis was: histiocytosis Langerhans, Hand-Schuller-Christian form. The patient receives treatment according to the LCH III Protocol. Langerhans cell histiocytosis is a disease that raises some difficulties in diagnosis. Our case has some particularities, not being a typical case for the Hand-Schuller-Christian clinical form.
Key words: histiocytosis, child, rare disorder

Langerhans cell histiocytosis is a group of idiopathic disorders characterized by the proliferation of cells of the mononuclear phagocyte system and the dendritic cell system.[2] The working group of the Histiocyte Society has divided histocytic disorders into 3 different groups: (1) dendritic cell histiocytosis, (2) erythrophagocytic macrophage disorders, and (3) malignant histiocytosis.[3] LCH belongs in group 1 and encompasses a number of diseases. These disorders are also rare, the prevalence being of 5 cases in 200.000 children per year. The incidence of LCH is greater in males than in females, with a male-to-female ratio of 2:1.[4,5] From the clinical point of view there are 3 forms of histiocytosis: acute, disseminated called Abt-Letterer-Siwe (it usually affects children under 2 years of age), eosinophilic granuloma and the intermediate clinical form called Hand-Schüller-Christian disease is characterized by multifocal, chronic involvement and classically presents as the triad of diabetes insipidus, proptosis, and lytic bone lesions. The pathogenesis of LCH is unknown.[5] Many years it was considered as a disorder at the border between benign and malignant and still it isn't established whether this is a reactive or neoplastic process. The etiology of LCH remains unknown.LC proliferation may be induced by a viral infection, a defect in intercellular communication (T cell–macrophage interaction), and/or a cytokine-driven process mediated by tumor necrosis factor, interleukin 11, and leukemia inhibitory factor.[2,5]

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We present the case of 15 years old boy with a symptoms combined: some that belong to one of the clinical forms and some of the other. These facts led to some difficulties in establishing the diagnosis.

The patient was treated initially in the dermatology department for a cutaneous eruption that was considered the symptom of the Darier disease. He had this eruption since 2007 and he was receiving local treatment for the skin lesions. The character of the eruption was: red papules with crusts isolated and confluated localized on the scalp, anterior and posterior thorax, abdomen and intertriginous areas. At the beginning the patient didn't seem to have other subjective complaints. It was performed also skin biopsy twice during his hospitalization in the dermatology department but the results were inconclusive. After receiving local treatment for the skin lesions the evolution of the eruption seemed to improve.

As the patient came for re-evaluation at some point it was noticed subfever, low apetite so he was sent for a pneumoftiziology exam. After taking chest x-rays (front and profile) that showed the presence of an opacity and pulmonary fibrosis in the median lobe of the right lung it was performed also a Quantiferon TB testing that turned out positive. It was initiated the treatment with isoniaside due to suspicion of primary TB infection based on x-ray and lab findings. At this point the parents mentioned that the adolescent has an increased intake of fluids especially during the night (fact that it didn't seam important to the family until the late findings). The endocrinologic exam raises the

suspicion of diabetes insipidus. So the patient is sent in our clinic for further investigations, the new suspicion based on the clinical and explorations data being of histiocytosis Langerhans. The clinical exam at the admittance of the boy in our clinic didn't find any other signs besides the skin eruption (Figure 1) (no enlarged lymph nodes or hepatosplenomegaly).

The biological exams showed the presence of inflammatory syndrome, didn't show significant modifications of the complete bloodcount, the renal and liver function are normal. The volume of urine in 24 hours is increased (3500ml), as well as the intake of fluids, the urine specific gravity is low (1002), the osmolarity of the urine is 74 mOsm and urinary sodium is lower than 80 ml/l. According to these data the diagnosis of diabetes insipidus was established.

The abdomen ultrasound showed no modifications. The chest x-ray revealed reticulate aspect of the lungs (Figure 2). The skeletal x-rays showed no lisis zones of the bones, only an osteocondensate lesion of the right femur (1/0,7 cm).

Also at the functional exploration of the lungs it was discovered severe respiratory disfunction: mixed type (restrictive and obstructive). The ECG and heart ultrasound: tachycardia (130/min), tricuspidian and mitral insufficiency.

The morphopathological exam of the skin biopsy revealed suggestive aspect for histiocytosis and the imunehistochemistry findings were: CD1a which is the specific marker for histiocytosis and protein S100 that is also a marker for this disease.

Based on the clinical exam and the biological and functional data the positive diagnosis was: histiocytosis Langerhans, Hand-Schuller-Christian form.

The patient receives treatment according to the LCH III Protocol.[1] It was chosen the treatment in the group 1 arm B which means multisystem risk patients. The treatment consists in corticotherapy(continuous oral prednisone-40 mg/mp daily as a 4 week corse, tapering over a period of 2 weeks), Vinblastine 6 mg/mp i.v. bolus, day one for 6 weeks, Methotrexate 500mg/mp 24 hours infusion day 1 of week 1, 3, 5. He also is treated with DDAVP (desmopressin) for the diabetes and the pneumoftiziolog recommended to keep treating him with isoniaside.

The evolution of the patient was good: the skin lesions began to fade (the crusts disappear and they are

Figure 1. Clinical aspects of the skin eruption in our patient

Figure 2 Chest x-rays of the patient front and profile

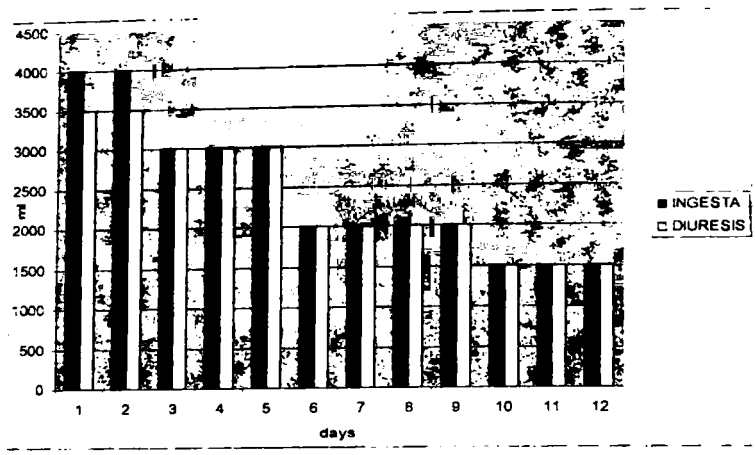

Figure 3. Graphic of the fluids intake and diuresis of the patient

pale), also the diabetes is under control, the intakeof fluids as well as diuresis diminished (Figure 3).

DISCUSSIONS

Langerhans cell histiocytosis is a disease that raises some difficulties in diagnosis being a rare disorder that has multiple symptoms as the result of the multiple organ disfunctions. The skin lesions often mislead the clinician and if the patient receives corticotherapy (the suspicion of diagnosis being dermatosis) the eruption diminishes because it is also the treatment for histiocytosis.

Even if it isn't quite established if this is a neoplastic disease the general studies that were developed found that the evolution is better under chemotherapy. [2,5]

Our case has some particularities due perhaps to the long evolution without specific treatment. The eruption that is characteristic for the acute disseminated form Abt-Letterer-Siwe, and also the respiratory disfunction is an aspect of the same clinical form. But the fact that he developed diabetes insipidus set the diagnosis of Hand-Schuller-Christian form because this disfunction is one of the elements of the triad that is specific for this clinical form.

The other elements of the triad (proptosis, lytic bone lesions) are not present but we think that diabetes insipidus is perhaps the most important aspect of the organ involvement in this clinical form.

The other elements that made us exclude the other clinical form are: the fact that Abt-Letterer-Siwe form appears usually in children under two years of age and it is a form with rapid severe evolution without treatment because of the multiple organ disfunctions.

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A case of superior recurrent digestive bleeding by esophageal varix secondary to portal vein thrombosis

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In children, thrombosis of portal vein may be caused by neonatal omphalitis, umbilical vein catheterisation, portal phlebitis, hypercoagulability, compression by ganglions, etc. or it may be the result of tumoral invasion. Clinic: abdominal recurrent pain, splenomegaly. Chronic thrombosis is accompanied by cavernous transformation and the development of perispleenic and left-gastric collateral circulation - esophageal varix. Ecographic findings: the absence of extrahepatic portal vein visualisation and multiple venous canals in liver's hilum. Doppler examination: turbulences, with the maintenance of venous flux and respiratory variations. The sensitivity of ecographic diagnostic is 100%. Splenomegaly, and the thickening of small omentum or splenorenal shunts may be present

Case presentation: a 2,6 year-old girl, with premature (28 gestational weeks) birth, in pelvic presentation, 700g weight, Apgarscore 5/1';6/5', with hylan membrane disease, necessiting umbilical vein catheterisation. From the age of 7 months she presented many hospitalizations for superior digestive haemorrhage, esophageal varix, thrombosis of portal vein, cavernoma and portal hypertension were diagnosed. Varices were alcohol-sclerosated, subsequently (at the age of 2) their legation was performed. Abdominal ecography: hepatomegaly, portal cavernoma, gallblader - callous walls. Endoscopy: only the esophagoscopy could be done: esophageal varix degree III-IV, violet coloured, imminence of bleeding. She followed treatment with Beta-blockers, and after the haemorrhage with Controloc, VitaminK and erythrocyte mass (for anaemia). Conclusions: in case 3 of a child with degree IV prematurity and multiple superior digestive hemorrhages, the performing of an endoscopy is necessary. When there are esophageal varices, their legation and sclerosis are just paleative procedures until the spleno-renal shunt is performed.

Key words: varix, esophagus, portal Vein,child.

Digestive bleeding is blood lost at digestive tracts level, acute or chronic; the following can be manifestations of digestive bleeding: haematemesis, melena, hematochezia or wastage of red blood in faeces, acompanied by severe clinical simptoms.[4,9,10]

Haematemesis represents wastage of blood by oral route. The blood from the vomiting liquid can be red or with "coffe ground" aspect. The red colour of blood from vomiting liquid shows the short-time contact between blood and gastric juice, fact that appear in proximal bleedings, or in digestive abundant fast bleedings.[4,9,10]

The "coffe ground" vomitings appear in case of slow bleedins (esophageal, stomach or duodenal bleedings) and they show that the blood suffered a gastric digestion.[9,10,13]

Melena represents the wastage of black faeces by digestive bleedings upon the ileocecal valve.[4,9,13]

Esophageal varix represent the most frequent cause of superior digestive bleeding, having esophageal seat, the dilation of veins in esophageal walls apear in situations with portal hypertension with prehaepatic, haepatic, or posthaepatic causes.[5,12]

At children, thrombosis of portal vein may be caused by neonatal omphalitis, after umbilical vein catheterisation, portal phlebitis, dehydration, hypercoagulability, compression by ganglions, or it may be the result of tumoral invasion.[3,6,8]

Clinic children with thrombosis of portal vein accuse abdominal recurrent pain and they present splenomegaly. Chronic thrombosis is accompanied by cavernous transformation and the development of perispleenic and left-gastric collateral circulation - esophageal varix.[8,14]

Ecographic findings are the absence of extrahepatic portal vein visualisation and multiple venous canals in liver's hilum. Doppler examination: turbulences, with the maintenance of venous flux and respiratory variations. The sensitivity of ecographic diagnostic is 100%. Splenomegaly may be present, and the thickening of small omentum or splenorenal shunts.[1,2]

Endoscopic aspect of esophageal varix has a great value in profilactic treatment in this patients. They appear as some winding cordons beginning from the superior cardial region where they have the maxim diameter, nd they go till 25 cm from dental arcade.[5,9,11]

Dagradi classifies esophageal varix in five degree, accordind to the diameter.[5,9]

-degree 1: small varix, blue or red colour, under 2 mm diameter, and 2mm upon the mucosal plan, appearing only during the Valsalva manoeuvre

-degree 2: blue varix, between 2 and 3mm diameter

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-degree 3: blue lofty varix, between 3 and 4mm diameter

-degree 4: blue winding varix, over 4mm diameter

-degree 5: varix obstruating esophageal lumen, or red small varix upon grey-blue varix

Varix have bleeding risk if they have red spots between 2-3 mm diameter at the level of roofing mucosa, if they have black spots (red spots in evolution) and if there are varix upon varix - which look like whip marks - in portal hypertension (with imminence of bleeding).[5,11]

In phase of varix bleeding, some drugs such as vasopressin, somatostatin or metoclopramid or a tampon - balloon could be used as teatment.[6,14]

The varix bleedings can be stopped in emergency with a gastric balloon, after 6-24 hours the sclerotherapy being performed.[5,12]

The differential diagnostic of digestive superior bleeding (esophageal varix) in children includes: esophageal and gastic peptic ulcer disease, esophagitis, gastritis, hyatal hernia, esophageal tumors, foreign body, Malory Weiss syndrome, trombocitopaenia, false haematemesis (reccurent alimentar vomiting with blood marks alimentar vomiting coloured by medication (iron, bismuth), swollow epistaxis, haemoptizia, oro-dental bleedings, swollowed blood from breast lesions in neonates).[3,9,10]

The treatment of digestive superior bleeding includes: hospitalisation, because of the unforesseable evolution of bleedings, prevention of shock (ventilation, blood volume relocation), vasopresor amine such as adrenalin, and other drugs like steroids, lactulosis, neomicyne, novoseven.[4,10,13,14]

As a peculiarity is the treatment of esophageal varix in context of portal hypertension: compression with esophageal balloon (Blakemore) with vasopresin i.v., oral propranolol, eviction of aspirin and other anti-inflammatory drugs.[4,10,]

CASE PRESENTATION

We present the case of a 2,6 year-old girl browght in emergency in our department, in several times, for episods of vomiting with fresh blood, medium-large quantity, diagnosed with esophageal varix, portal vein thrombosos, portal cavernoma and portal hypertension, secondary to umbilical vein catetherisation during neonatal period.

Patient G.D.N., from Mures, rural provenience, was many times hospitaliyed in our center, since 2006.

Antecedents: patient femine gender, aged 2 years and 6 month, from the second pregnancy of her mother, pregnancy with phisiological evolution until 28 weeks of gestation, when, afirmative after a great phisical effort of mother began the premature delivery; faetus in pelvine presentation, weight 700g, Apgar score 5/1';6/5', with hylan membrane disease, necessiting umbilical vein catheterisation.

From the age of 7 months she presented many hospitalizations for superior digestive haemorrhage. It were diagnosed esophageal varix degree III, thrombosis of portal vein, cavernoma and portal hypertension in 2006. The varix were alcohol-sclerosated, subsequently (at the age of 2, in 2007) their legation being performed for a new episode of superior digestive bleeding.

Clinic she presented relative good general health status, pale skin, red pharinx, collateral visible circulation at thoracic and abdomen level, laterocervical adenopathy, pulmonary and cardio-vasculary normal findings, without sensitivity at abdominal palpation, liver 2cm under right costal rebord.

Hemoleucogram: detects anaemia (haemoglobin 6.3- 8,4-9 g/dl, haematocrit 22.3%, eritrocitar medium volume 73.6 fl.) ESR 12 mm/h.

Biochemistry: normal values.

Ionogram: normal values, Iron 6,3 µmol/l.

Coagulograma: normal values.

Abdominal ecography (Figure 1) shows liver with homogenous echostructure and normal ecogenity, 92,5/50 mm, normal echographic aspects of kidneys and spleen; in conclusion: hepatomegaly, portal cavernoma, gallblader with callous walls. (Figure 2)

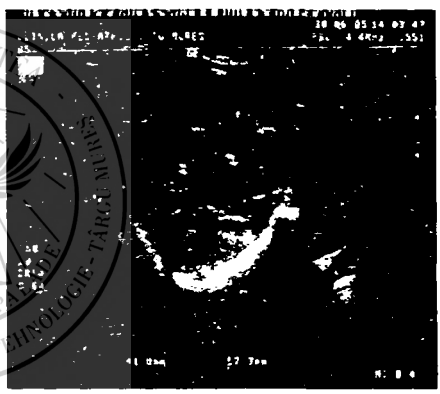

Figure 1. Portal cavernoma – ultrasound aspect

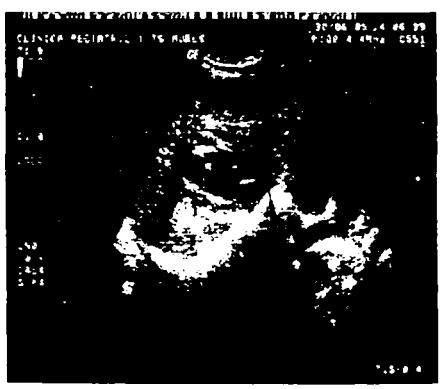

Figure 2. Portal cavernoma– Doppler ultrasound aspect

Digestive endoscopy: only the esophagoscopy could be done, which showed esophageal varix degree III-IV (Figure 3), with violet colour and imminence of bleeding (Figure 4).

Figure 3. Esophagoscopy – esophageal varices IV degree, violet aspect

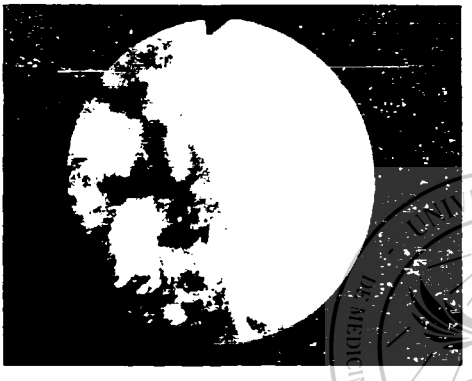

Figure 4 Upper gastrointestinal endoscopy – esophageal varices with imminence of break

Corroborating anamnestic data and clinico-paraclinic aspects the diagnostic of Superior Digestive Bleeding, Esophageal varix, Portal vein thrombosis, Portal cavernoma, Portal hypertension, Anaemia and Acute angina was established.

Treatment: She has treatment with Beta-blockers, which was pursued during hospitaliyation, associated with Controloc, VitaminK; because of the severe anaemia (after-bleeding) the patiend necessitated a transfusion. She also received antibiotic treatment for respiratory acute disease. Then the case was sent to another center in order to be established the subsequently therapeutic attitude, the legation and sclerosis of varix being just paleative procedures until the spleno-renal shunt can be performed.

DISCUSSIONS

We are presenting the case of a girl known with esophageal varix and portal hypertension, with cavernous transformation of portal vein, with recurrent superior digestive bleedings, often during in context of a small local trauma or associated to a respiratory acute disease.

Is spite of the fact that she presents thrombosis of portal vein, our patient does not accuse abdominal recurrent pain and she does not present splenomegaly, but we can see that cavernous transformation and development of perispleenic and left-gastric collateral circulation resulting esophageal varix are the consequences of chronic thrombosis of portal vein.

CONCLUSIONS

In front of a child with prematurity degree IV and multiple superior digestive bleedings, performing a digestive endoscopy is extremly important.This must be made in emergency room, in order to iluminate the diagnostic. When there are esophageal varix, their legation and sclerosis are just paleative procedures until the spleno-renal shunt is performed.

Until the surgical solution in this type of cases it is vital to prevent the recurrency of bleeding by eviction of trauma – even of small local trauma which can be produced by food, associated with recongnizing and precocious treatment of any acute disease which could produce and maintain bleedings, even bz vomiting perception or cough effort.

We must never forget that an abbundant superior digestive bleeding in child can be caused by the break of esophageal varix secondary to portal hypertension in context of a portal vein thrombosis (appeared early after-birth).

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Studies on the enzymatic activity of *Genista tinctoria L* extracts

Amelia Tero-Vescan, Alexandrina Oşan

Aim of study: to evaluate the activity of PPO and AO in correlation with the concentration of polyphenols and ascorbic acid.

Materials and methods: Genista tinctoria L was harvested in Călimani Mountains in June 2007. Enzymatic activity was determined by Margna method, polyphenol compounds were determined by a spectrophotometric method from the Romanian Pharmacopoeia 10th edition and ascorbic acid was determined by a spectrophotometric method with αα – dipyridyl.

Results and conclusions: PPO activity decreases in dry plant by 54.84%, while polyphenol content increases by 181.81%. AO activity decreases in dry plant by 72.71%, which is correlated with the ascorbic acid content decrease (71.06%).

Key words: PPO, AO, polyphenols, ascorbic acid, *Genista tinctoria L.*

It is well-known that, no matter the evolution stage, all living entities are governed by enzymatic activity. Knowing the enzymatic activity of plants is important from a physiologic and physiopathology point of view. That is how the increasing interest in the enzymatic activity determination can be explained.[2,5,6,7,9,10]

There are many studies on the enzymatic activity modifications during the growth, ripping and conservation of different types of fruits and vegetables.[1] Variation in the activity of: pectinase, polygalacturonidase, clorophylase, acid phosphatase, L-asparaginase, amylase, glucose-6-phosphate dehydrogenase, hexokinase, aldolase, piruvate-, malate, succinate-dehydrogenase, ribonuclease, peroxidase, polyphenoloxidase, etc, was determined while growing, ripping and conservation of fruits and vegetables. Starting from these considerations, we studied the activity of polyphenoloxidase (PPO) and ascorbicoxidase (AO) from Genista tinctoria L extracts.

The content of phenol substances and ascorbic acid which represent the substrates of these enzymes was also determined. PPO (E.C.1.10.3.1.) and AO (E.C.1.10.3.3.) are oxidoreductases, copper dependent, very well-spread in plants.

PPO is an oxidoreductase that catalyzes the aerobic oxidation of phenol compounds to o-quinone. O-quinones are unstable substances and tend to condense and turn their color into brown. That is how the darkening of bruised vegetal tissues can be explained. Quinone stability decreases with the increase of molecular weight and the number of phenol groups. PPO can also use as substrate esters of hydroxycinnamic acid, mono- and biflavones. Then obtained products can oxidize flavonic glycosides and anthocyanic compounds that are not directly attacked by PPO.[1,2]

AO is also an oxidoreductase, copper dependent that can be found in cytoplasm tied to the cellular membranes. It oxidizes ascorbic acid to dehydroascorbic acid. The inverted reaction is catalyzed by glutathionereductase (NADPH dependent) and dehydroascorbatreductase.[1,4]

Genista species contain isoflavonoids and flavonoids (genistein, genistin, daidzein, daidzin, luteolin, luteolin-5-glycosid, luteolin-5,7-diglycosid, formononetin, ononin, etc), quinolizidine alkaloids (anagyrine, cytisine, N-methyl cytisine, sparteine, isosparteine) and small amounts of volatile oil, fat oil, mucilaginous substances, tanants.[3]

MATERIALS AND METHODS

-Genista tinctoria L was harvested in June 2007 from an area in Căliman Mountains. From the fresh and dry plant we obtained 20% extracts in phosphate-citrate buffer (pH=8). Tampon was selected by preliminary researches that used different types of solutions, but in the end phosphate-citrate pH= 8 buffer was selected.

-Enzymatic activity of PPO and AO was determined by Margna method.[8] PPO in the presence of oxygen oxidizes the substrate (pirocatequine) to o-benzoquinone that oxidizes the excess of ascorbic acid.

The excess of ascorbic acid was determined by an iodatometric method. For AO determination samples were made without pirocatequine, and the same quantity of ascorbic acid was added. 5 determinations were made

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for each sample type. Enzymatic activity was expressed in mg ascorbic acid/min/100 g plant.

-Determination of total polyphenols was made by Cynarae folium method from FR X.[11] 20% extractive solutions were made from Genista tinctoria L in 50% ethanol.

Results were expressed in mg caffeic acid/100 g fresh and dry plant and were determined from the medium calibration curve A=73.125(±8.23) (c+0.0852 (±0.0119). The calibration curve was made between $4x10^{-4}$-$32x10^{-4}$ mg/ml domain with caffeic acid, N=5 concentration points, with 3 determinations for each point of concentration.

-Ascorbic acid determination was made by a spectrophotometric method.[8] This method consists in reduction of Fe^{3+} to Fe^{2+} by ascorbic acid and Fe^{2+} forms with αα'dipyridyl a pink photometrable complex. 20% extractive solutions were made from fresh and dry plant in metaphosphoric acid 2%. Results were expressed in mg ascorbic acid/100 g fresh and dry plant and were determined from the medium calibration curve A = 0.0431(±0.0097)*c+0.0213(±0.0240). The calibration curve was made on 0.25-10.00 mg/ml ascorbic acid domain, N=10 concentration points, with 3 determinations for each point of concentration.

RESULTS AND DISCUSSION

Weight loss by drying values are presented in Table I. Enzymatic activity of PPO and AO and poliphenol and ascorbic acid content were determined in fresh and dry plant extracts.

The results obtained in fresh plant extracts were transformed into mg /100 g dry plant by calculating the weight loss through drying.

Results obtained in PPO and AO determination from Genista tinctoria L extracts are illustrated in Table II and Table III.

Medium activity of PPO for fresh Genista tinctoria L extracts is 39.42±1.58 mg ascorbic acid/min/100 g plant. In extracts obtained from dry plant the medium activity of PPO is 17.08±1.57 mg ascorbic acid/min/100 g plant which represents a decrease of 54.84% compared with the fresh plant.

Medium activity of AO for fresh Genista tinctoria L extracts is 30.27±0.79 mg ascorbic acid/min/100 g plant. In extracts obtained from dry plant the medium

Table I – Determination of fresh plant weight loss by drying

Sample no	g fresh plant	g dry plant	Weight loss by drying %
1	10.4235	3.0125	71.09
2	10.0231	2.9899	70.16
3	9.9981	2.9567	70.42
Medium loss %			70.55

Table II. PPO activity values from fresh and dry Genista tinctoria L extracts

PPO in fresh plant extracts		PPO in dry plant extracts	
Sample no.	Enzymathic activity mg ascorbic acid/min/100 g plant	Sample no.	Enzymathic activity mg ascorbic acid/min/100 g plant
P1	40.48	P1	17.80
P2	40.48	P2	16.00
P3	38.72	P3	16.04
P4	36.96	P4	16.04
P5	40.48	P5	19.52
Result	39.43±1.58	Result	17.10±1.57
CV%	3.99	CV%	9.16
Differenece % between fresh and dry plant		-54.84	
		p<0.001	

Table III. AO activity values from fresh and dry Genista tinctoria L extracts

AO in fresh plant extracts		AO in dry plant extracts	
Sample no.	Enzymathic activity mg ascorbic acid/min/100 g plant	Sample no.	Enzymathic activity mg ascorbic acid/min/100 g plant
P1	31.68	P1	8.94
P2	29.92	P2	7.22
P3	29.92	P3	8.94
P4	29.92	P4	8.94
P5	29.92	P5	7.22
Result	30.27±0.79	Result	8.26±0.94
CV%	2.60	CV%	11.41
Differenece % between fresh and dry plant		-72.71	
		p<0.001	

Table IV. Total polyphenols values in extracts obtained from fresh and dry Genista tinctoria L

Polyphenols in fresh plant extracts		Polyphenols in dry plant extracts	
Sample no.	mg caffeic acid/100 g fresh plant	Sample no.	mg caffeic acid/100 g dry plant
P1	0.12	P1	0.25
P2	0.11	P2	0.29
P3	0.09	P3	0.36
P4	0.13	P4	0.31
P5	0.12	P5	0.32
Result	0.11±0.02	Result	0.31±0.04
CV%	13.30	CV%	13.19
Differenece % between fresh and dry plant		+181.81%	
		p<0.001	

Table V. Ascorbic acid values in extracts obtained from fresh and dry Genista tinctoria L

Ascorbic acid in fresh plant extracts		Ascorbic acid in dry plant extracts	
Sample no.	mg ascorbic acid/100 g fresh plant	Sample no.	mg ascorbic acid/100 g dry plant
P1	3.47	P1	1.09
P2	3.61	P2	0.98
P3	3.47	P3	1.00
P4	3.98	P4	1.05
P5	3.24	P5	1.02
Result	3.56±0.27	Result	1.03±0.04
CV%	7.64	CV%	4.30
Differenece % between fresh and dry plant		-71.06	
		p<0.01	

activity of PPO is 8.25±0.94 mg ascorbic acid/min/100 g plant which represents a decrease of 72.71% compared with the fresh plant.

Results obtained for total polyphenols determination from Genista tinctoria L extracts are presented in Table IV.

In fresh plant concentration of polyphenols expressed as caffeic acid was 0.11±0.02 mg/100g and 0.31±0.04 mg/100 g for dry plant extracts. The increased values of polyphenols in dry plant can be due to the fact that PPO is more active in fresh plant and can oxidize a part of the polyphenols.

Results obtained for ascorbic acid determination from Genista tinctoria L extracts are presented in Table V.

In fresh plant medium concentration of ascorbic acid was 3.56±0.27 mg/100g and 1.03±0.04 mg/100 g for dry plant extracts. There is a -71.06% decrease of the ascorbic acid from fresh to dry plant extracts. This is because ascorbic acid is very unstable and can be oxidized by AO and atmospheric oxygen.

CONCLUSIONS

Determinations made on fresh Genista tinctoria L extracts suggest that enzymatic activity of polyphenoloxidase and ascorbicoxidase are similar, but they significantly decrease in dry plant extracts. Polyphenol content is increased in dry plant extracts while ascorbic acid content is significantly decreased.

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Comparative statistical study of drug dissolution profiles as function of temperature, stirring speed and medium pH

Szende Vancea[1], Silvia Imre[2], Gabriella Donáth- Nagy[1], B. Tőkés[1]

In vitro dissolution may be relevant to the prediction of drug in vivo performance. In order to evaluate correctly dissolution tests' results experimental conditions such as dissolution medium pH, temperature, stirring speed should be carefully chosen. In the present study we examined these factors in terms of physical chemistry. Dissolution tests were performed using immediate release oral tablets which contain 20 mg fosinopril sodium. The paddle method was applied. Dissolution medium consisted of ultrapure water or buffer solution with pH 1.2 and pH 6.8. The stirring speed was varied between 0-100 RPM, the temperature set to 25, 50 or 37 °C. At several time points samples were taken. For determination of samples' fosinopril content a liquid chromatographic-UV detection method was used. The appropriate dissolution profiles- fosinopril concentration (mg/mL) and dissolved substance (Q%) versus time were plotted. The processing of raw data was based on different statistical considerations. The obtained results identify the optimal rate of dissolution conditions for fosinopril tablets.
***Key words**: dissolution testing, temperature, agitation, pH*

Drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Because of the decisive importance of the first two of these steps, in vitro dissolution may be relevant to the prediction of in vivo performance. Based on this general consideration, in vitro dissolution tests for immediate release solid oral dosage forms, such as tablets and capsules, are used to assess the lot-to-lot quality of a drug product; and guide development of new formulations and ensure continuing product quality and performance after certain changes, such as in the formulation or in the manufacturing process.[2,4,10,11]

In order to evaluate correctly dissolution tests' results experimental conditions such as dissolution medium pH, temperature, stirring speed should be carefully chosen. In the present study we examined these factors in terms of physical chemistry. The processing of raw data was based on different statistical considerations.

MATERIALS AND METHOD

Dissolution tests were performed using immediate release oral tablets which contain 20 mg fosinopril sodium (Monopril®, Bristol-Myers Squibb, Batch 8G32164). The paddle method in USP Apparatus 2 (Erweka) was applied.[3] Dissolution medium consisted of 900 mL ultrapure water (Direct-Q Millipore) or buffer solution with pH 1.2 - 250 mL NaCl (Merck KGaA) 0.2 M + 425 mL HCl (Merck KGaA) 0.2 M diluted to 1000 mL with ultrapure water - and pH 6.8 - 250 mL KH_2PO_4 (Merck KGaA) 0.2 M + 112 mL NaOH (Merck KGaA) 0.1 M.[1] The stirring speed was varied between 0-100 RPM, the temperature set to 25, 50 or 37 °C. After 5, 10, 15, 20, 30, 40 minutes samples of 1 mL were taken. For determination of samples' fosinopril content, a liquid chromatographic-UV detection method was used.[5-9] The analyte was quantified automatically by the instrument data system using the external standard method. Calibration was performed using singlicate standards. Stock solution of fosinopril sodium was prepared by dissolving appropriate quantities of reference substance (Hetero Drugs Limited, Batch FLWS/08) in 10 mL methanol (Merck KGaA). Six calibration working solutions of 2 - 40 mg/mL fosinopril were then obtained by diluting specific volumes of stock solution with ultrapure water. The concentration domain was chosen according to the expected maximum analyte concentration in the samples, 22.22 mg/mL (20 mg fosinopril sodium in 900 mL medium). The calibration curve model was determined by the least squares analysis. The HPLC system was an 1100 series model (Agilent Technologies) consisted of a binary pump, an in-line degasser, an autosampler, a column thermostat, and an UV detector. Chromatographic separation was performed at 35°C on a Zorbax SB C8 Solvent Saver Plus (Agilent), 100 x 3 mm, 3.5 mm column. The mobile phase consisted of a mixture 45:55 v/v of water containing 15 mM H_3PO_4 (85% Merck KGaA) and acetonitrile (Merck KGaA). The pump delivered the mobile phase at 1 mL/min. The samples were stored in the autosampler at 20 °C, and 10 ml was injected into the LC-UV system. The detection of the analyte was achieved at 205 nm. Chromatograms were processed using QuantAnalysis software, dissolution and

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statistical data were calculated using Microsoft Excel and Origin softwares.

The most important functions used were:

-Boltzmann function:

$$y = \frac{A_1 - A_2}{1 + e^{(x-x_0)/dx}} + A_2$$

where

x0 = center

dx = width or time constant

A1 = initial Y value

A2 = final Y value

-Logistic function:

$$y = \frac{A_1 - A_2}{1 + (x / x_0)^p} + A_2$$

where:

x0 = center

p = power

A1 = initial Y value

A2 = final Y value

-Hill function:

$$y = V_{max} \frac{x^n}{k^n + x^n}$$

where:

Vmax = maximum velocity

k = Michaelis constant

n = cooperative binding sites

RESULTS AND DISCUSSIONS

We have performed the dissolution tests on different medium pH, temperature and stirring speed values and determined the fosinopril content of withdrawn samples by the described LC-UV method (Figure 1).

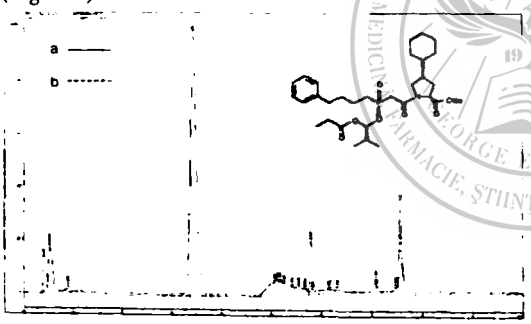

Figure 1. Typical chromatograms of a- fosinopril sodium (t_R= 3. 4 min), and b- blank

Than we constructed the appropriate dissolution profiles- fosinopril concentration (μg/mL) and dissolved substance (Q%) versus time. After the selection of different type of curve fitting functions we calculated the curve parameters according to the values of temperature, pH and agitation. As far as we are aware, this is the first published statistical evaluation of the dissolution profile of fosinopril which takes in consideration the recommandations of FDA guidance regarding model dependent approaches.[10]

The results show that the experimental data distribution can be described with an S-shaped (sigmoidal) curve in all of the cases. We have selected the statistical evaluation methods presented in the introduction according to this distribution. Figure 2 compares various statistical procedures for dissolution profile under constant conditions (water, 25 °C, 50 RPM), the speed of release proves that the parameters put in different perspectives the studied phenomenon.

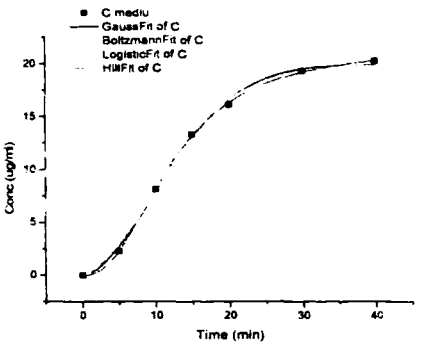

Figure 2. Growth/ Sigmoidal curves on dissolution profile (water, 25 °C, 50 RPM) of 20 mg fosinopril sodium tablets (different type of fitting functions: Gaussian, Boltzmann, Logistic, Hill)

The increase of temperature and of stirring speed (Figure 3 and 4) increases the release speed. Comparison of the curve slopes reflects qualitatively this statement too. The increase is mainly accentuated until the temperature of 37 °C, and the stirring speed of 75 RPM.

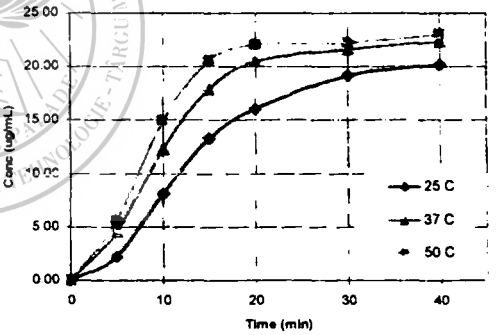

Figure 3. The influence of temperature on fosinopril Na dissolution

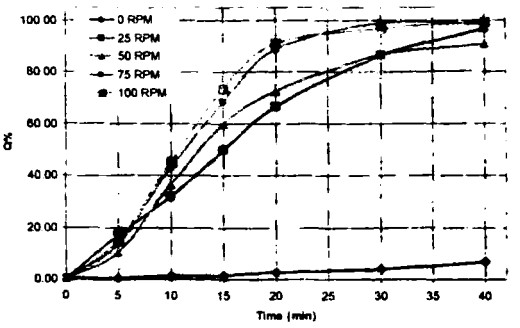

Figure 4. The influence of stirring speed on fosinopril Na dissolution

These changes can be quantified with the used statistical parameters k, p dx, independent of each other. Numerical values can be seen as proportional quantities with the dissolution speed, so that they refer directly to the phenomenon studied (Table I and II).

The c^2 values present in the tables below underline that the variable parameters are in close relation to each other.

The dissolution medium pH's effect on the dissolution speed (Figure 5) shows the expected monotonic relationship between them.

Table I. Curve parameters as function of temperature

		25 C	37 C	50 C
Hill	χ2	0.02078	0.18907	0.32639
	R 2	0.99957	0.99606	0.99283
	Vmax	21.52	23.13	23.27
	k	12.36	9.21	7.76
	n	2.35	2.43	2.74
Logistic	χ2	0.02075	0.1845	0.32304
	R 2	0.99968	0.99767	0.99618
	A1	-0.01	0.12	0.10
	A2	21.53	23.11	23.26
	x0	12.36	9.25	7.79
	p	2.35	2.46	2.75
Boltzmann	χ2	0.35277	0.10603	0.06479
	R 2	0.99455	0.99866	0.99923
	A1	-3.13	-3.06	-2.16
	A2	20.00	22.05	22.59
	x0	10.59	8.28	7.45
	dx	5.37	4.13	3.16

Table II. Curve parameters as function of stirring speed

		0 RPM	25 RPM	50 RPM	75 RPM	100 RPM
Hill	χ2	0.00518	0.28062	0.02078	0 52828	0.47156
	R 2	0.98279	0 9941	0.99957	0.99096	0 9916
	Vmax	26570.38	30.46	21.52	23.34	23.01
	k	35534.77	21.39	12.36	11 34	10.63
	n	1 44	1.46	2.35	2.59	2.68
Logistic	χ2	0.00499	0.27031	0.02075	0.50247	0.43574
	R 2	0 98384	0.99574	0.99968	0.99386	0 99465
	A1	0.02	0.18	-0.01	0.27	0 33
	A2	18801.88	29.97	21.53	23 25	22.90
	x0	22719.20	21.07	12.36	11.44	10.75
	p	1.49	1 50	2.35	2 67	2.77
Boltzmann	χ2	0.00388	0 0721	0.35277	0.15293	0.05514
	R 2	0 98743	0 99886	0.99455	0 99813	0.99932
	A1	-0.69	-7.88	-3.13	-2.16	-1 83
	A2	197.73	22.90	20.00	22 11	21.92
	x0	189.41	10.24	10.59	10.56	10.09
	dx	33.44	9 78	5.37	4 44	4 02

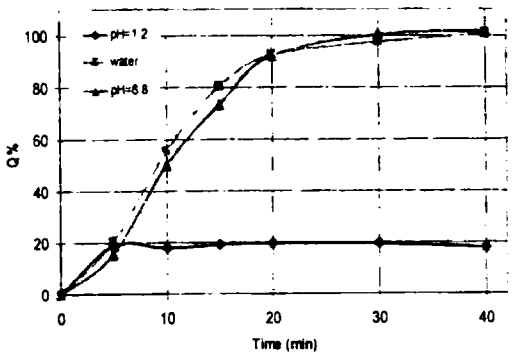

Figure 5. The influence of dissolution medium pH on fosinopril Na dissolution

Table III. Curve parameters as function of dissolution medium pH

		pH=1.2	water	pH=6.8
Hill	$\chi 2$	0.04065	0.18907	0.33433
	$R\,2$	-0.619	0.99606	0.99412
	$Vmax$	4.22	23.13	23.70
	k	0.13	9.21	10.47
	n	1.07	2.43	2.50
Logistic	$\chi 2$	0.04065	0.1845	0.33042
	$R\,2$	0.9839	0.99767	0.99607
	$A1$	2.647E-06	0.12	0.11
	$A2$	4.22	23.11	23.67
	$x0$	0.13	9.25	10.50
	p	1.07	2.46	2.53
Boltzmann	$\chi 2$	0.04172	0.10603	0.31276
	$R\,2$	0.98348	0.99866	0.99628
	$A1$	-12.45	-3.06	-2.90
	$A2$	4.19	22.05	22.53
	$x0$	-0.98	8.28	9.49
	dx	0.90	4.13	4.46

While at pH = 1.2 the release is small-scale and constant over time, at pH 6.8, the dissolution profile takes the shape of a sigmoid mentioned above. Of course, this step markes out convincingly clear from the values in the table too (Table III).

The obtained results identify the optimal rate of dissolution conditions (temperature, stirring speed, pH) for fosinopril tablets.

CONCLUSIONS

We found that the rate of dissolution and active substance release is closely linked to the stirring speed, temperature and acid-base properties of the dissolution medium. The dissolution profile data (the quantity of released active substance per unit of time) can not be considered as absolute value, since slight changes in the experimental conditions strongly influence the delivery kinetics. These studies can be used with good results for comparison of two products, as well as for comparison of different batches or to optimize the manufacturing process. The results are comparable to processes occuring in the organism and the circumstances of the in vivo processes were aproximated as much as possible.

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Healing after periapical endodontic surgery. A 2 year retrospective study

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The aim of this study was to report a 2 year follow-up of a group of patients, whose teeth were treated for periapical lesions of endodontic origin using periapical resection, evaluating the prognosis using healing classification of different authors. 94 patients (127 teeth) with chronic periapical lesions were included. Patients were recalled within the 6, 12, 24 months time period and radiographs were taken. Clinical signs and symptoms were recorded. Of the 127 teeth evaluated according to the classification introduced by Molven et al., the overall success rate was 83.3% (complete plus incomplete healing). Considering the evaluation criteria of Mikkonen et al. the success rate was: success in 75%, improvement in 14%, and failure in 11%. The success rate was greater in the no-post group. Finally, it was evaluated if the tooth was functional (remained in place) or not and the results are: 94,07% functional and 5.92% not functional.
Key words: periapical surgery, endodontic surgery, success

Surgical endodontics is a reliable method for the treatment of teeth with periapical lesions that do not respond to conventional root canal treatment[15,8,5] or when orthograde retreatment is not feasible.[7,1] The success rate in periapical surgery varies between 37%[25] and 91%.[27] In the last years, successful outcomes have been reported in over 80 % of cases.[10,3,27] This high success may be due to modern surgical techniques[6], microsurgery instruments[17,18,2], ultrasonic retrotips[20,19,13,24], magnifying devices[4], and improved root-end filling materials.[26] The aim of this study was to report a 2 year follow-up of a group of patients whose teeth were treated for periapical lesions of endodontic origin using periapical resection, evaluating the prognosis using healing classification of different authors.

MATERIAL AND METHOD

A clinical follow-up study was carried out over a period of 2 year in 136 patients with chronic periapical lesions. The patients were treated with periapical surgery (91 female and 45 male) in a Private Practice, by one surgeon (KE). The inclusion criteria for patients in the study where: 1.) need for apicectomies in case of endodontic treatment failures and 2.) at least 12 months follow up after treatment. Data were collected using a protocol for each patient, by clinical and radiological examination and the patients were asked to fill in a form. 33 patients were excluded for lack of follow-up. 9 teeth in 9 patients were extracted during the procedure because of vertical root fracture or lateral perforation; these were eliminated from the study. Thus a total of 127 teeth (94 patients) were included. Patients were recalled within the 6, 12, 24 months time period. 33 patients were eliminated because they did not attend recalls. All the patients included came to at least one of the three control appointments. Among the 127 teeth 85 were anterior, 38 premolars and 4 molars. There were analyzed the quality of the root end filling and the presence of a root canal pivot. The lesions affected one or more apices and varied in maximum diameter between 3 and 12 mm. Clinical signs and symptoms were recorded preoperatively and also at each follow-up appointment. The presence of clinical signs and symptoms after the first review were classified within the unsuccessful case group.

Surgical procedures. All procedures were performed by one surgeon. Vestibular intrasulcular incision that excludes the dental papilla (papillary-based incision) or a submarginal incision (Ochsenbein-Luebke flap) was used. Approximately 2-3 mm of root apices were resected and the presence of guttapercha and/or cement in the canal was confirmed, if not, root end preparation was used with an ultrasonic technique (Mectron micropiezo s, with the R3 tip for retrograd root end preparation). The ultrasonic unit was used on medium power setting. The canals were prepared to a depth of 2-3 mm using specially designed tips. A oxifosfat cement (Adhesor – Spofa Dental) mixed with iodoform, or MTA (Pro Root MTA, Dentspy, Tulsa, OK) was used as the root end filling material. No space making or space maintenance biomaterials were placed in the wound. Bleeding was induced in the field if it was absent at the end of the filling and cleaning procedures.[18] Non absorbable silk (4-0) (D-tek) was used for suturing.

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There was no antibiotic prescription before and after the surgery. Nonsteroidal anti-inflammatory drugs for 1-2 days were administered to limit postoperative pain.

Radiographs were taken prior to surgery, after surgery and at each follow up appointment. All radiographs were examined twice (interval of almost 20 days) under 2 x magnification and assigned to the appropriate category at the corresponding time. Multirooted teeth were categorized on the basis of the root with the least favourable result. All the radiographs used to monitor healing of each case were evaluated according to the classification introduced by Molven et al.[22,23] In this way, each tooth was classified into one of the following categories: 1.) complete healing; 2.) incomplete healing; 3.) uncertain healing; 4.) unsatisfactory healing (failure).

The success of periapical surgery was also evaluated by clinical criteria of Mikkonen et al.[21] These are: 1) success: when there is no pain, swelling or fistula, 2) uncertain healing: radiographic evidence of bone destruction and presence or not of symptomatology, and 3) failure: when there is bone destruction, root resorbtion and symptomatology. Teeth with clinical signs and symptoms reported at each of the control visits were categorized as failures. On the contrary, the first two categories were always associated with no clinical signs or symptoms.

Finally, it was evaluated if the tooth was functional (remained in place) or not.[10,11,12] All cases were divided into three groups based on the type of teeth, i.e. anterior, premolars and molars. The presence of posts was taken into account. For this reason the sample was further divided into two subgroups, teeth with (post group) or without posts (no-post group). The teeth were divided into anterior, premolar and molar, and percentages were calculated to assess differences between the groups. The sample was then divided into post and no-post groups as reported above. In addition, to simplify the comparison between post and no-post groups, complete and incomplete healing were added to the 'successful' cases and the other two categories (uncertain and unsatisfactory healing) to the 'unsuccessful' cases.

RESULTS

178 teeth in 136 patients were treated surgically. 9 teeth in 9 patients were extracted during the procedure because of vertical root fracture or lateral perforation; they were eliminated from the study. 33 patients were eliminated because they did not atrend regular follow-up appointments. Therefore, a group of 94 patients and 127 teeth were examined. Of the 127 teeth evaluated at the end of the study (after 6 months), 89 teeth (70%) healed with complete bone filling of the surgical cavity and adequate radiological periradicular architecture, 22 (17.3%) had an apical scar, only 7 teeth (5.5%) had uncertain healing and 9 (7%) were classified as failures. The overall success rate was 83.3% (complete plus incomplete healing). A more detailed description of the data is presented in Table I.

A cast post in the canals was seen in 35.4% of the teeth. For this reason, the frequency distribution of healed cases was analysed by dividing the teeth into two groups: post and no-post. As shown in Table II and Table III, 45 teeth with cast posts included six failures (13.3%), and 82 teeth with no post included 2 failures (2.4%).

Table I. Distribution of the teeth included in the study and outcomes

Type of healing	6 months	12 months	24 months	Total (No./%)
Complete	5	15	5	25 (55.5)
Incomplete	2	3	2	7 (15.5)
Uncertain	-	4	3	7 (15.5)
Failure	4	-	2	6 (13.3)

Table II. Results: post (teeth with cast post inside)

	Complete No. of teeth (%)	Incomplete No. of teeth (%)	Uncertain No. of teeth (%)	Failure No. of teeth (%)	No. of teeth (%)
Anterior	63 (74.1)	8 (9.4)	10 (11.7)	4 (4.7)	85 (66.9)
Premolar	26 (68.4)	6 (15.7)	3 (7.8)	3 (7.8)	38 (29.9)
Molar	1 (25)	2 (50)	0 (0)	1 (25)	4(3.14)
Total	90 (70.8)	16 (12.5)	13 (10.2)	8 (6.2)	127 (100)

Table III. Results: no post group (teeth filled only by cement and/or gutta-percha)

Type of healing	6 months	12 months	24 months	Total (No. /%)
Complete	20	32	13	65 (65)
Incomplete	4	3	2	9 (9)
Uncertain	3	3	-	6 (6)
Failure	2	-	-	2 (2)

Further analysis was performed adding the first and the second categories (complete and incomplete healing) to the success case group and with the other two categories in the failure group. The results are reported in Table IV and demonstrate only a slight difference at the end of the observation period. Most of the successful cases were considered healed within the first year of surgery (65%).

Table IV. Outcome at 24 months of the two groups of teeth, with posts in root canals and without

	Post	No post
Success	71.2%	90.2%
Failure	28.8%	9.8%

None of the cases that healed in the short term failed at a later date.

Considering the evaluation criteria of Mikkonen et al.[21] the success rate was: success in 75%, improvement in 14%, and failure in 11%.

Finally, it was evaluated if the tooth was functional (remained in place) or not and the results are: 94,07% functional and 5.92% not functional.

DISCUSSION

Studies on the outcome of periapical surgery have reported variable results, ranging from a 30-80% success rate.[14,16] However, these studies differed in sample size, surgical technique, type of teeth, type of root end filling material and radiographic evaluation criteria. Recently, some longitudinal studies reported a higher success rate in periapical surgery of teeth not responding to orthograde endodontic treatment.[27,28]

From the results obtained in this study, we find the overall success rate in periapical surgery with our methods was about 83,3% considering the evaluation of Molven. When using the evaluation criteria of Mikkonen et al. the success rate was about: success in 75% and improvement in 14%.[21] The success rate was greater in the no-post group, proving that periapical surgery with retrograd filling has some difficulties, and the technichnique must be improved. Dorn & Gartner[9] reported a 75% success rate for amalgam root end filling and over 90% for a zinc oxide-reinforced material. In contrast, some authors had a lower success rate using these materials.[12] Sumi et al.[27] reported a success rate of 92.4% using only microhandpieces for root end cavity preparation. In the majority of the cases, the time to successful radiological healing was estimated to be within 1 year, and further investigations should be carried out to determine other key factors influencing the quality of the healing process.

CONCLUSION

Periapical resection is a predictible method. In the majority of the cases, the time to successful radiological healing was estimated to be within one year, and further investigation should be carried out to determine other key factors influencing the quality of healing process.

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Invasive cervical resorption: predisposing factors and treatment options

Monica Pop[1], Cristina Bică[2], Adriana Monea[1]

The purpose of our paper is to present the predisposing factors and treatment possibilities for invasive cervical resorption of teeth, with emphasis on the prognosis. We examined 24 teeth in this condition in 24 patients with an average age of 34 years, in class 1-4 of resorption according to the Heithersay classification. The treatment was nonsurgical in 37,5% of the cases and endodontic prior to surgery in 62,5% of the cases.

Our results showed that the predisposing factors were: orthodontic treatment (39%), trauma (29%), surgery in the cemento-enamel region (20%) and bleaching of teeth (12%). The rate of success for nonsurgical treatment was 72, 3%; in teeth class 3 and 4 success was recorded in only 33,3% of the cases, 1 had a continuous resorption, 1 had an apical periodontitis and 2 were extracted due to fracture.

We came to the conclusions that invasive cervical resorption must be under periodic radiological examination to make sure that the resorptive process has stopped. These teeth present a high risk to fracture and patients must be instructed how to prevent plaque formation in order to avoid periodontal complications.

***Key words**: cervical resorption, osteoclasts, resorption lacunae*

Cervical resorption also known as external inflammatory resorption is a relatively rare condition that has been a subject of debate for clinicians for over a century. It is a rather uncommon condition, not-easily recognized that can occur subsequent to orthodontic tooth movement, orthognathic or other dentoalveolar surgery, bleaching and a wide variety of traumatic injuries of the teeth.[1,2,3]

Invasive cervical resorption describes a problem that can occur in any permanent tooth and is characterized by a massive loss of tooth structure; destruction of coronal enamel and dentin is followed by proliferation of a highly vascularized tissue that becomes visible through the thin enamel, aspect called *pink spot*.

As its designation implies, this begins in the cervical area of the tooth, below the epithelial attachment. The damaged area of the root surface can be very small and the resorbing cells may penetrate the tooth through these spaces, causing the resorptive process to spread into the dentine.[2]

At first, the resorption will be limited outside the pulp chamber and root canal due to the protective qualities of the predentine and it will have an irregular form, inside the tooth. Because of this extension pattern, it is also called external, internal or invasive cervical resorption. A clinical classification of cervical resorption had been developed by Heithersay et al.[7,8] for research purposes and also to provide a clinical guideline for cases presenting this pathology:

-Class 1 - small invasive resorption lesion near the cervical area with shallow penetration into the dentine;

-Class 2 - well defined resorption lesion that had penetrated close to the coronal pulp chamber but shows little or no extension into the radicular dentine;

-Class 3 - a deeper invasion of the dentine, not only involving the coronal part but extending into the coronal third of the root;

-Class 4 - denotes a large resorption process that had extended beyond the coronal third of the root.

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The purpose of our paper is to present the predisposing factors and treatment options for teeth in this condition, with emphasis on the prognosis.

MATERIAL AND METHOD

During October 2005- October 2008 we examined 24 cases of teeth with invasive cervical resorption (4 molars, 11 canines, 6 prime premolars, 3 second premolars) in 24 patients with an average age of 34 years (19-62 years). In 9 cases the resorption lacunae were diagnosed by a routine radiographic examination, as the patients were under orthodontic or periodontal treatment and in 15 cases the patients had severe pain due to pulp inflammation. According to the classification system developed by Heithersay et al (1999) 9 teeth were in class 1, 9 teeth in class 2, 4 teeth in class 3 and 2 teeth in class 4.

As nonsurgical treatment for class 1 and 2, topical application of an aqueous solution of trichloroacetic acid 90%, followed by curettage and restoration of the defect with a composite resin or glass-ionomer cement was recommended[7] Within class 1 defects our treatment consisted in the exposure of the defect, curettage of all granulation tissue and restoration with glassionomer cement. Within class 2 defects we performed nonsurgical treatment restoring the tooth after careful removal of the granulation tissue but in some cases we preferred an endodontic treatment before restoring the tooth, in order to avoid late complications (inflammation or necrosis of the pulp).

In order to perform resorptions in class 3 and 4 we chose the surgical treatment combined with endodontics, which involved periodontal flap reflection, curettage of granulation tissue, restoration of the defect with composite resin or glassionomer cement and repositioning of the flap in the original position. The endodontic treatment was completed before the final restoration of the defect with composite resin or glassionomer cement. In two cases within class 4 we were not able to realize the root filling prior to surgery as we could not identify the root canal in the apical third of the root. In these cases a flap was raised, the root canal was obturated and during the same meeting the resorptive process was closed. Antibiotics and anti-inflammatory drugs were recommended for 7 days, when the sutures were removed.

The follow-up up consisted of retroalveolar radiographs every 6 months, periodontal probing to evaluate the aspect of the root filling and the status of periodontal tissues. A final evaluation was performed in 21 cases because 3 teeth had been lost due to fracture.

RESULTS

Orthodontics was the most common etiological factor of invasive cervical resorption in the studied group (9 teeth, 39%), followed by trauma (7 cases, 29%), surgery in the cementoenamel region (5 cases, 20%) and bleaching of teeth (3 cases, 12%).

In the group of class 1 and 2 teeth the follow-up showed good results, with light discoloration of the restoration and no history of dental pulp inflammation. Regarding defects included in class 3 and 4 we started with the endodontic treatment and the surgical exposure of the defect during the next meeting. At this point a full thickness flap was raised, the defect was cleaned and the tooth restored with glassionomer cement.

We present the treatment of two cases of class 3 and 4 teeth where the predisposing factors of invasive cervical resorption was orthodontic treatment and periodontal surgery (Fig. 1A-D, 2A-D).

Figures 1 A-D. Tooth 13 with class 3 cervical resorption in a 34 year old patient, with a history of orthodontic treatment. Preoperative radiography shows a large defect on the distal aspect of the tooth, which starts in the cervical area and extends in the coronal third of the root. After the endodontic treatment the resorptive area was exposed and restored with composite resin.

Figure 2 A-D. Tooth 44 with class 4 cervical resorption which penetrates deep into the middle third of the root, in a 62 year old patient with a history of periodontal surgery. It was impossible to performer the endodontic treatment without raising a periodontal flap, because we could not penetrate the apical part of the root without direct view. The defect was restored with glassionomer cement.

The rate of success for nonsurgical treatment was 72,3% but in 5 teeth (37,5%) the resorption was present again after a period of 6-12 months. In class 3 and 4 teeth success was achieved in only 33,3%, 1 had a continuous resorption, 1 had an apical periodontitis and 2 were extracted due to fracture. These results indicate an outcome of satisfactory treatment only for class 1 and 2 teeth and a poor prognosis for those in class 3 and 4.

DISCUSSION

Mineralized tissues of permanent teeth are not normally resorbed, because they are protected inside the root canal by predentine and odontoblasts and outside the root by precementum and cementoblasts[9] If these structures become mineralized or in case of precementum they are mechanically damaged, osteoclast-like cells will colonize these surfaces and resorption will start.[14] However, the resorbing cells require continuous stimulation during phagocytosis, without this the process is limited and will need repair with formation of cementum – like tissue. This type of resorption is called transient root resorption and has no clinical importance, the defects being too small to be observed, even radiographically.[6,11]

Root resorption which starts at the root surface may be sustained by mechanical irritation, increased pressure in the tissue or infection outside the tooth or in the root canal.[10] Mechanical stimulation of osteoclasts is seen in root-fractured teeth where the sharp edges of the root fragments are selectively resorbed[5]. Pressure resorption in the permanent dentition may be seen during orthodontic treatment, usually in the form of apical resorption and shortening of the roots. Root resorption sustained by infection is the most important clinical condition from the endodontic point of view and it can occur inside the root canal (internal resorption) or on the root surface (cervical resorption) depending on weather the microbial stimuli come from inside the tooth or the gingival sulcus.[3]

At first, the resorbtive process will not penetrate to the pulp because of the protective qualities of the predentin, but extend around the root canal, which in time may become an endodontic problem. In early radiographs the root canal walls are clearly seen, which help us to make the difference between external and internal root resorption.[15]

Cervical resorption is usually asymptomatic and in most of the cases will be diagnosed during a routine radiographic examination. However, the patient may complain of a pulp origin pain if the resorption has perforated to the root canal and exposed the pulp. If this resorptive process reaches a supragingival area of the crown, a well vascularized granulation tissue may be visible through the enamel and the patient will present a so-called pink spot.

The treatment should aim at the inactivation of all resorbing tissue and reconstruction of the resorptive defect either by placement of a filling material or by use of biological systems.[17] The most effective approach is to expose the resorption lacuna surgically, to remove the granulation tissue and to fill the defect with a suitable restorative material (amalgam, glassionomer cement, composite resin).

Periodontal reattachment cannot be performed with amalgam and composite resin and it is unlikely to be done with glassionomer cement, but there is experimental evidence to suggest that this might be possible by using Mineral Trioxide Aggregate.

Teeth in which perforation to the root canal occurred need endodontic treatment, which should be made prior to the surgical exposure of the resorption lacuna.[12,13] This has the advantage that the resorption with small external openings may be cleaned and obturated from the root canal, however follow-up examinations are important to make sure that the process has been arrested.[15,17]

CONCLUSIONS

1. Teeth with invasive cervical resorption must be under periodic radiological examination, to make sure that the resorptive process had stopped.

2. Due to important loss of dental hard tissues from the cervical area of the tooth, these teeth present a high risk of fracture and their resistance is poor.

3. On the surface of the restorative material a long junction of epithelium is formed, an area of low resistance against infection, and a periodontal pocket can easily appear. Therefore, the patient must be instructed to prevent the formation of dental plaque.

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The efficiency of subgingival curettage in the removing of dental calculus deposit

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The success of periodontal therapy depends on the possibility of the clinician to detect and to wholy clean the supra- and subgingival calculus deposits.
Aim. We have made this study in order to evaluate the efficiency of the subgingival curettage in the removal of the subgingival dental calculus deposits. Material and method. We selected and analyzed 35 single- and multi-roothed teeth. After apreciating the parodontal status we switch to removing the supra- and subgingival deposits of calculus. To apreciate the effectiveness of subgingival curettage we practiced two marks, in the axial end, for each tooth. After the extraction the teeth were examined with the optical stereomicroscope. Results and discussions. Of the 35 teeth on wich we have practiced closed subgingival curettage, 24 teeth (representing approximately 68%) presented residual subgingival calculus deposits. In apreciating the number of areas which had radiculare remaining subgingival calculus, of the 150 areas examined we found that 51 (representing 34% of them) had deposits on the areas between the two marks. Other authors have made similar observations. Conclusions. Removing all of the calculus after closed subgingival curettage is virtually impossible. The efficiency of subgingival curettage is higher in the mesial surfaces compared to other dental surfaces, both single-and multi-rooted teeth.
***Keywords**: periodontal therapy, subgingival curettage, subgingival calculus deposits*

Dental calculus (tartar) is an organo-mineral complex adherent to the dental surface or other solid structures in the oral cavity, resulting from the mineralization of the bacterial plaque.

The presence of dental tartar was invariably associated with periodontal disease. As the surface of calculus is covered by a layer of anmineralised plaque , it was difficult to establish if dental calculus 'per se' has a destructive effect on the periodontal tissue.

Convincing experimental investigations have established that dental tartar is not pathogenic in the absence of bacterial plaque.[6,9,10,1] The outburst of periodontal disease is carried out by bacterial plaque and the maintenance of the inflammation is due to the tartar which always has a young bacterial plaque on its surface.

The etiologycal significance of dental calculus in parodontal disease is supported by the following facts:

- dental calculus iritates the gum with its presence,
- is permeable and can store toxic products, including endotoxins,
- it is covered by the bacterial plaque and the correlation between the plaque and the appearance of gingivitis and periodontitis is certain[8].

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Mandell and Gaffar state that the dental calculus, due to its porosity and retention of bacterial antigens, maintains the inflammatory process of the marginal periodontal tissue initiated by bacterial plaque.

From the presented facts we can conclude that the dental calculus is a favorable factor for the periodontal inflammation because it exercises a mechanic role in maintaining the bacterial plaque in close contact, even irritating, trough its increase in volume, with marginal periodontal tissues and stops, in some areas , the access of artificial cleaning and self-cleaning methods of the bacterial plaque.

Starting with these observations we can conclude that the success of periodontal therapy depends on the possibility of the clinician to detect and to wholy clean the supra and subgingival calculus deposits.

We have made this study in order to evaluate the efficiency of the subgingival curettage in the removal of the subgingival dental calculus deposits.

MATERIAL AND METHOD

For the purpose of this study we selected and analyzed 35 single- and multi-roothed teeth belonging to patients who presented themselves at the Clinic of Odontology and Periodontology Targu-Mures, in different forms of periodontal disease.

The main criterions of selection of teeth to inculde in this study were:

- unfavorable prognosis for maintaining in the dental arch, which allow us to extract them;
- the existence in the odonto-parodontal units of periodontal pockets with depth = 3.5 mm;
- have not benefited of periodontal treatment in the past year.

After apreciating the parodontal status for each dento-periodontal unit we switch to removing the supragingival deposits of calculus. The closed subgingival curettage was performed with Gracey curettes and did not exceed 15 minutes for a tooth.

To apreciate the effectiveness of subgingival curettage in the radiculare areas that are involved in the treatment of chronic marginal periodontitis and because all teeth had more advanced forms of periodontal disease, we practiced two marks, in the axial end, for each tooth . The first mark was done *in vivo* right in the gingival edge with a conical diamond cutter of turbine. The second mark was done *in vitro* after the tooth extraction, also with a conical diamond cutter of turbine at a distance of approximately 3.5 mm apical from the first. After the extraction the teeth were washed and maintained in a solution of 10% formalin until there examination with the optical stereomicroscope.

RESULTS

Of the 35 teeth that have received closed subgingival curettage, when examined with the optical stereomicroscope, 24 teeth (representing approximately 68%) presented residual subgingival calculus deposits (Table I).

Table I

	Number of teeth	Number of teeth with residual calculus
Single-rooted teeth	28	19
Multi-rooted teeth	7	5
Total	35	24

At the single-rooted teeth we detected residual subgingival calculus deposits on 19 teeth (representing 68% of them and 54% of all the examined teeth).

Remaining subgingival calculus deposits were identified on 5 (representing 71% of them and 14% of all the examined teeth) of the 7 multi-rooted teeth reviewed.

In apreciating the number of areas which had radiculare remaining subgingival calculus, of the 150 areas examined we found that 51 (representing 34% of them) had deposits on the areas between the two marks.

Of the 112 tooth surfaces of single-rooted teeth examined 32 (representing 29% of them and 21% of the total surfaces examined) had subgingivale residual deposits of calculus (Table II), the rest being free of tartar (Figure 1).

Table II.

	Number of tooth surfaces	Number of tooth surfaces with residual calculus
Single-rooted teeth	112	32
Multi-rooted teeth	38	19
Total	150	51

Figure 1

On multi-rooted teeeth we detect subgingival residual calculus on 19 (representing 50% of them and 13% of the total dental surfaces examined) of the 38 dental surfaces examined (Table II). The 51 tooth surfaces that had subgingival residual calculus were divided like this: 6 mesial surfaces (representing 12%), 14 distal surfaces (representing 27%), 14 buccal surfaces (representing 27%) and 17 oral surfaces (representing 34%).

Of the 32-tooth surfaces of single- rooted teeth that had subgingival residual calculus deposit , 4 were mesial surfaces (representing 13% of them and 8% of all the sufaces with residual tartar) (Figure 2), 10 distal surfaces(representing 31 % of them and 19% of allthe sufaces with residual tartar), 8 buccal surfaces (representing 25% and 16%) and 10 oral surfaces (representing 31% and 19%).

Figure 2

The 19 surfaces of multi-rooted teeth with subgingival residual calculus were: 2 mesial surfaces (representing 11% of them and 4% of allthe sufaces with residual tartar), 4 distal surfaces (representing 21% of them and 8% of all the surfaces with residual tartar),

6 buccal surfaces (representing 31% of them and 11% of all the surfaces with residual tartar) (Figure 3) and 7 oral surfaces (representing 37% and 15%).

Figure 3

DISCUSSIONS

The complete removal of calculus is a primary part of achieving a "biologically acceptable" tooth surface in the treatment of periodontitis. The results of this study that tell us that 68% of teeth examined, and 34% of tooth surfaces examined had residual calculus deposits allow us to say that removing all of the calculus after closed subgingival curettage is virtually impossible.

Other authors have made similar observations. Caffesse et al[2] proposed to themselves to evaluate the effectiveness of subgingival curettage, with or without flap surgery, in relation with the calculus removal. The results of this study showed that both techniques increase the percentage of subgingival surfaces free of calculus and scaling following the reflection of a flap aided calculus removal in pockets 4mm and deeper.

A study made by Chan et al[3] was aimed to appreciate residual calculus deposits after a single episode of subgingival curettage. After the root instrumentation, the 30 teeth were extracted and viewed with the stereomicroscope. The percentage of surfaces which had remaining calculus was 41%, demonstrating the impossibility of the operator to detect and remove all subgingival tartar.

Anderson et al[1] evaluated the effectiveness of subgingival curettage comparing the effect of single instrumentation to the effect of three instrumentations. The authors concluded that subgingival curettage removed dental calculus, but not entirely. The results demonstrated no significant difference in the effectivness of calculus removal between single and multiple episodes of subgingival curretage.

Kepic et al[5] evaluated the effectiveness of closed subgingival curettage performed on 31 teeth, of which 17 were treated with hand instruments and 14 of them with an ultrasonic instrument. After treatment the teeth were extracted and preparated for light microscopic evaluation. Twelve of the 14 teeth treated by ultrasonic and 12 of the 17 treated by hand instruments retained calculus. The two types of instruments had comparable results, but hand instrumentations had appeard to be more effective then ultrasonics in removing cementum from proximal surfaces. The results of this study indicate that complete removal of calculus from a root surface is rare.

The use of the periodontal endoscope resulted a significant improvement in calculus removal during subgingival curettage, which was most evident in deeper periodontal pockets (Geisinger et al[4]).

Michaud et al[7] showed that the use of the endoscope as an adjunct to traditional subgingival curettage provided no significant improvement in calculus removal in multi-rooted molar teeth.

CONCLUSIONS

1. The periodontal pockets containe subgingival calculus deposits wich maintaine the inflammatory process of the marginal periodontal tissue.

2. Removing all of the calculus after closed subgingival curettage is virtually impossible.

3. On the multi-rooted teeth there is a higher incidence of calculus deposits remaining after closed subgingival curettage than on single-rooted.

4. The surfaces of single-rooted teeth can be accesed easier than those of multi-rooted teeth in subgingival calculus removal.

5. The efficiency of subgingival curettage in the removal of subgingivale calculus deposits is higher in the mesial surfaces compared to other dental surfaces, both single-and multi-rooted teeth.

6. Using the open approach of subgingival curettage, due to direct visual inspection, the efficiency in removing calculus in increased compared to closed subgingival curettage, in periodontal pockets with depth greater than 3.5 mm.

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