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Предменструално дисфорично разстройство

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Premenstrual Dysphoric Disorder

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РЕЗЮМЕ:

Предменструалното дисфорично разстройство (ПДР) е тежка форма на предменструален синдром (ПМС), който се отнася към група от менструално свързани разстройства, който засягат приблизително 40% от жените. ПМС се разглажда, като комплексно психоендокринологично разстройство, което засяга емоционалното и физическо благосъстояние на жените. Симптомите на ПМС се разгръщат по време на лутеалната фаза на менструалния цикъл и започват да отзвучават с началото на менструацията или малко след това. Приблизително 5% от жените с ПМС страдат от ПДР, по- дезадаптиращата и по-тежката форма на ПМС, която е хронично състояние, изисква лечение когато се появи и доминиращи в клиничната картина са афективните симптоми. Диагнозата трябва да бъде направена на базата на попълнен ежедневен пациентски симптоматичен

ABSTRACT

Premenstrual dysphoric disorder (PMDD) is a severe form of premenstrual syndrome (PMS), which refers to a group of menstrually related disorders that are estimated to affect up to 40 % of women. PMS is now viewed as a complex psychoneuroendocrine disorder that is known to affect women's emotional and physical well-being. PMS symptoms accur during the luteal phase of the menstrual cycle and remit with the onset of menstruation or shortly afterward. Approximatly 5 % of women with PMS suffer from PMDD, a more disabling and severe form of PMS which is a chronic condition that necessitates treatment when it occurs and mood symptoms predominate. The diagnosis should be made on the basis of a patient-completed daily symptom calendar and the exclusion of the other medical disorders. Available treatments include lifestyle modifications and medication. For some women, the symptoms of PMDD can

календар и да се изключат други соматични заболявания. Известните лечения включват, промяна в стила на живот и медикаменти. При някой жени симптомите на ПДР могат да продължават до менопаузата. Симптомите на ПДР са толкова тежки, че често нарушават способността на жената да функционира нормално в ежедневния и живот.

КЛЮЧОВИ ДУМИ: Предменструално дисфорично разстройство, Предменструален синдром, Менструален цикъл, Психоендокринно разстройство

last until menopause. The symptoms of PMDD are so severe that they often disrupt a woman's ability to function normally in her daily life.

KEY WORDS: Premenstrual dysphoric disorder, premenstrual syndrome, menstrual cycle, psychoneuroendocrine disorder

INTRODUCTION

Most women of reproductive age have some physical discomfort or dysphoria in the weeks before menstruation. It's estimated that as many as 2 of every 4 menstruating women have experienced some form of premenstrual syndrome. Symptoms are often mild, but can be severe enough to substantially affect daily activities. They tend to recur in a predictable pattern. About 5–8% of women thus suffer from severe premenstrual syndrome (PMS) also meet criteria for premenstrual dysphoric disorder (PMDD). Although more than 100 premenstrual symptoms have been described, common complaints include mood and behavioural symptoms: irritability, tension, depressed mood, tearfulness, and mood swings, a feeling of being out of control, abdominal pain, are the most distressing, but somatic complaints, such as breast tenderness and bloating, can also be problematic. The clinicion should try to group the various symptoms into clusters (e.g. mood, cognitive, physical symptoms and social consequences). Yonkers KA, et al.

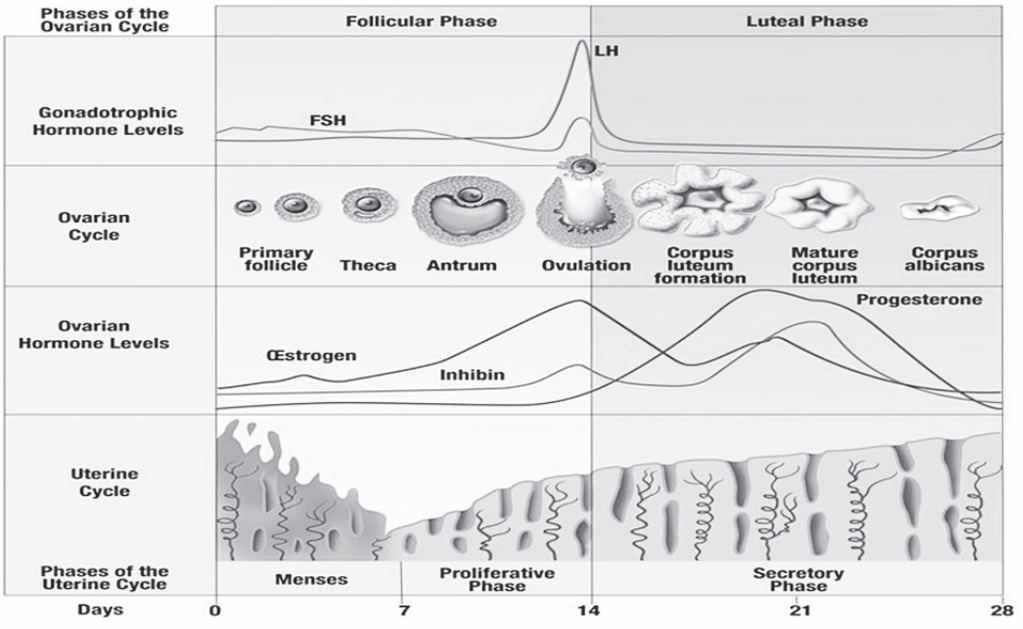
PMDD is a more severe premenstrual condition that affects about 1,8-5,8% of women during their reproductive years. These symptoms may cause them to avoid friends or relatives during the week before their period. Most researchers consider PMDD a type of mood disorder. The symptoms of PMDD appear regularly at some time after a woman ovulates in the middle of her monthly cycle. Symptoms generally get worse in the week before her period and then disappear during menstruation. The symptoms have occurred in most of the menstrual cycles during the past year and must interfere significantly with work, school, social activities, or relationships. The intensity and/or expressivity of the accompanying symptoms may be closely related to social and cultural background characteristics of the affected female, family perspectives, and more specific factors such as religious beliefs, social tolerance, and female gender role issues. Nevertheless, frequency, intensity, and expressivity of symptoms and helpseeking patterns may be significantly influenced by cultural factors. Premenstrual symptoms can begin at any age after a woman begins to menstruate. Some women report that symptoms worsen when they are in their 30s; others associate the onset of symptoms with a reproductive event, such as a baby's birth or surgery for tubal ligation. Premenstrual symptoms do not occur when a woman is pregnant, breast-feeding (at least during the first few months before menstrual cycles begin again), and after menopause. Therefore, it appears PMDD symptoms can only occur when a woman is having menstrual cycles. Margaret L. Moline, PhD

Before a diagnosis of PMDD can be made the physician should rule out a wide range of medical problems. Depending on the patient's presentation, these may include anemia, autoimmune disorders, thyroid disorders, diabetes mellitus, chrotic fatigue syndrome, and endometriosis. psychiatric disorders, such as majior depression, dysthymia, bipolar disorder, generalized anxiety disorder, and panic disorder, also should be exclude, especially in

women with dysphopric mood symptoms. In women over the age of 40, PMDD should be distinguished from various perimenopausal symptoms, such as breast tenderness, headache, and sleep disturbances. PMDD also must be distinguished from certain other disorders that can be exacerbated during the late luteal or menstrual phase of the cycle: migraines, seizure disorder, irritable bowel syndrome, asthma, allergies. Edyta J. Frackiewicz and Thomas M. Shiovitz

Sometimes, symptoms peak is around the time of the onset of menses. Although it is not uncommon for symptoms to linger into the first few days of menses, the individual must have a symptom-free period in the follicular phase after the menstrual period begins **figure 1.**

Figure 1. ***Phases of the Ovarian Cycle (Fairfax Media 2017)***



Presence of physical and/or behavioral symptoms in the absence of mood and/or anxious symptoms is not sufficient for a diagnosis. Symptoms are of comparable severity (but not duration) to those of another mental disorder, such a major depressive episode or generalized anxiety disorder. In order to confirm a provisional diagnosis, daily prospective symptom ratings are required for at least two symptomatic cycles. Delusions and hallucinations have been described in the late luteal phase of the menstrual cycle but are rare. The premenstrual phase has been considered by some to be a risk period for suicide. DSM-5

PREVALENCE

Twelve-month prevalence of premenstrual dysphoric disorder is between 1.8% and 5.8% of menstruating women. The most rigorous estimate of premenstrual dysphoric disorder is 1.8% for women whose symptoms meet the full criteria without functional impairment and 1.3% for women whose symptoms meet the current criteria with functional impairment and without co-occurring symptoms from another mental disorder. DSM-5

ETIOLOGY AND PATHOGENESIS

According to Bio – Psycho – Social model of illnesses we can divide etiologic factors and pathogenesis of PMDD in to three groups **Table 1.**

Table 1. ***Bio-Psycho-Social Model of PMDD (Arabadzhiev, Madzhurova 2017)***

Biological factors:	Psychological factors:	Social factors:
- not specific genens; - higher risk if a woman's mother had the condition; - after infectious desease; - endocrine disturbance; - disregulation between serotonine and dopamine; - seasonal changes	- personality; - coping strategies; - women who work more mentally; - ability to manage with stress. - core believes.	- stress; - personal or interpersonal traumas; - sociocultural aspects of female sexual behaviour in general; - female gender role in particular.

DIAGNOSIS

Before the release of the fifth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), premenstrual dysphoric disorder (PMDD) has been classified in DSM-IV-TR as a Mood Disorder Not Otherwise Specified. According to DSM-IV-TR, 3-5% of women of menstrual age may suffer from the disorder. Of these women, 90.6% consider the symptoms to be normal (not pathological) and 18.7% seek professional help, although in some cases they receive an inadequate response. Almost 20 years of research, the disorder has now been recognized as a distinct diagnostic entity through its inclusion in the newly published DSM-5. **Table 2** Leire Aperribai, PhDa,*, Itziar Alonso-Arbiol, PhDb, Nekane Balluerka, PhD b, Laurence Claes, PhDc

According to Bio – Psycho – Social model of illnesses we can divide clinical presentation of PMDD in to three clusters **Table 3.**

DEVELOPMENT AND COURSE

Onset of premenstrual dysphoric disorder can occur at any point after menarche. Anecdotally, many individuals, as they approach menopause, report that symptoms worsen. Symptoms cease after menopause, although cyclical hormone replacement can trigger the re-expression of symptoms. DSM-5

DIFFERENTIAL DIAGNOSIS

Premenstrual syndrome. Premenstrual syndrome differs from premenstrual dysphoric disorder in that a minimum of five symptoms is not required, and there is no stipulation of affective symptoms for individuals who have premenstrual syndrome.

Dysmenorrhea. Dysmenorrhea is a syndrome of painful menses, but this is distinct from a syndrome characterized by affective changes. Dysmenorrhea begin with the onset of menses, whereas symptoms of premenstrual dysphoric disorder begin before the onset of menses.

Bipolar disorder, major depressive disorder, and persistent depressive disorder (dysthymia). PMDD has clear interval of at least 7–10 days during each menstrual cycle when the woman feels well mentally and physically. If a woman is depressed or anxious all month long, even if she feels worse premenstrually, it is more likely that she has another kind of mood problem (such as major depression) rather than PMDD. DSM-5

COMORBIDITY

A major depressive episode is the most frequently reported previous disorder in individuals presenting with PMDD. A wide range of medical (e.g., migraine, asthma, allergies, seizure disorders) or other mental disorders (e.g., depressive and bipolar disorders, anxiety disorders, bulimia nervosa, substance use disorders) may worsen in the premenstrual phase. DSM-5

TREATMENT

Treatment of PMDD includes both nonpharmacologic and pharmacologic therapies. Nonpharmacologic therapy includes aerobic exercise, consumption of complex carbohydrates and frequent meals, relaxation training, light therapy, sleep deprivation, and cognitive-behavioral therapy (CBT). The efficacy of lifestyle interventions (e.g, diet, exercise, and vitamin supplementation) and psychothera-

Table 2. ***Diagnostic Criteria DSM 5 (DSM 5, 2013)***

A. In the majority of menstrual cycles, at least five symptoms must be present in the final week before the onset of menses, start to improve within a few days after the onset of menses, and become minimal or absent in the week postmenses.
B. One (or more) of the following symptoms must be present:
1. Marked affective lability (e.g., mood swings: feeling suddenly sad or tearful, or increased sensitivity to rejection).
2. Marked irritability or anger or increased interpersonal conflicts.
3. Marked depressed mood, feelings of hopelessness, or self-deprecating thoughts.
4. Marked anxiety, tension, and/or feelings of being keyed up or on edge.
C. One (or more) of the following symptoms must additionally be present, to reach a total of five symptoms when combined with symptoms from Criterion B above.
1. Decreased interest in usual activities (e.g., work, school, friends, hobbies).
2. Subjective difficulty in concentration.
3. Lethargy, easy fatigability, or marked lack of energy.
4. Marked change in appetite; overeating; or specific food cravings.
5. Hypersomnia or insomnia.
6. A sense of being ovenwhelmed or out of control.
7. Physical symptoms such as breast tenderness or swelling, joint or muscle pain, a sensation of „bloating," or weight gain.

Note: The symptoms in Criteria A-C must have been met for most menstrual cycles that occurred in the preceding year.

D. The symptoms are associated with clinically significant distress or interference with work, school, usual social activities, or relationships with others (e.g., avoidance of social activities; decreased productivity and efficiency at work, school, or home).
E. The disturbance is not merely an exacerbation of the symptoms of another disorder, such as major depressive disorder, panic disorder, persistent depressive disorder (dysthymia), or a personality disorder (although it may co-occur with any of these disorders).
F. Criterion A should be confirmed by prospective daily ratings during at least two symptomatic cycles. (Note: The diagnosis may be made provisionally prior to this confirmation.)
G. The symptoms are not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication, other treatment) or another medical condition (e.g., hyperthyroidism).

Recording Procedures

If symptoms have not been confirmed by prospective daily ratings of at least two symptomatic cycles, „provisional" should be noted after the name of the diagnosis (i.e., „premenstrual dysphoric disorder, provisional").

Table 3. ***Bio-Psycho-Social Model of Clinical Presentation (Arabadzhiev, Madzhurova 2017)***

Biological (physical) symptoms:	**Psychological (mood) symptoms:**	**Social consequences:**
- breast tenderness; - bloating; - fatigue; - physical discomfort; - hypersomnia/insomnia; - swelling; - muscle and goint pain.; - headache; - constipation/diarrhea.	- dysphoria; - irritability, anxiety - tension; - depressed mood; - tearfulness; - mood swings; - anger, - food craving.	- avoid frienfs or relatives during the period; - interfere significantly with work, school, social activities; - interpersonal conflicts.

peutic interventions for PMDD remains unclear.

NONPHARMACOLOGIC THERAPY

Acupuncture and herbal medicine treatments for premenstrual syndrome and premenstrual dysphoric disorder showed a 50% or better reduction of symptoms compared to the initial state of the patients.

The relaxation response is a physiologic response that results in decreased metabolism, a lower heart rate, reduced blood pressure, a lower rate of breathing, and slower brain waves. The repetition of a word, sound, prayer, phrase, or muscular activity is required to elicit the relaxation response.

The light emitted by conventional fluorescent lamps is deficient in many of the colors and wavelengths of natural sunlight. The basis of light therapy is replacing such lamps with full-spectrum fluorescent lamps whose light (referred to as bright light) is more similar to sunlight. The effect of bright light was postulated to be mediated through the serotonin system.

Most patients with major depressive disorder respond to a night of total sleep deprivation. Because of the relation of this disorder to PMDD, treatments for major depressive disorder may also be effective for PMDD.

Cognitive therapy is based on the view that behavioral disorders are influenced by negative or extreme thought patterns, which are so habitual that they become automatic and are unnoticed by the individual. Cognitive treatment teaches patients ways of examining these negative patterns and replacing them with more adaptive ways of viewing life events. CBT for PMDD includes anger control, thought stopping, and reduction of negative emotions through cognitive restructuring. Thwe T Htay, MD

PHARMACOLOGIC THERAPY

Antidepressants that slow the reuptake of serotonin are effective for many women with PMDD. Options include selective serotonin reuptake inhibitors (SSRIs) such as citalopram (Cipralex, Essobel) and fluoxetine (Biflox, Prozac); the serotonin and norepinephrine reuptake inhibitor (SNRI) venlafaxine (Effexor), and Duloxetine (Dulsevia); and a tricyclic antidepressant that has a strong effect on serotonin, called clomipramine (Anafranil). Other types of antidepressants, which target neurotransmitters other than serotonin, have not proven effective in treating PMDD. This suggests that serotonin reuptake inhibitors work in some way independent of their antidepressant effect — but their mechanism of action in PMDD remains unclear. These drugs also alleviate symptoms of PMDD more quickly than depression, which means that women don't necessarily have to take the drugs every day. Instead, women can take them on an intermittent basis, also known as luteal-phase dosing because it coincides with the roughly 14-day span that begins just after ovulation and ends when menstruation starts. Intermittent dosing is sufficient for treating irritability or mood, but daily medication may be necessary to control somatic symptoms such as fatigue and physical discomfort. If anxiety or insomnia are the prevailing symptoms, a clinician may prescribe a benzodiazepine, such as alprazolam (Xanax), in addition to an SSRI or SNRI. Cunningham J, et al

One of the most common PMDD treatments is progesterone supplementation, but the studies consistently find no evidence that a deficiency of this hormone contributes to the disorder. The hormone therapies that do seem to work in PMDD act not by countering hormonal abnormalities, but by interrupting aberrant signaling in the hypothalamic-pituitary-gonadal circuit that links brain and ovaries and regulates the reproductive cycle. They are considered as second-line treatments for PMDD. Oral contraceptives have seldom been studied for this purpose, and it's not clear if they are effective. Another option is to inhibit ovulation with estrogen, which can be delivered via a skin patch or via a subcutaneous implant. Doses of estrogen tend to be higher than those prescribed for hormone therapy during menopause, but lower than those used for contraception in childbearing years. If estrogen is prescribed, it should be taken along with a progestogen to reduce risk of uterine cancer — except for women who have had a hysterectomy. Gonadotropin-releasing hormone (GnRH) agonists, which are usually prescribed for endometriosis and infertility, sup-

press the hormonal cycle — and may be helpful for women whose PMDD symptoms have not responded to other drugs. Examples of GnRH agonists include buserelin (Suprefact) and goserelin (Zoladex). But these agents can induce a menopausal state, triggering hot flashes and increasing risk of osteoporosis, so they are often supplemented with estrogen and a progestogen — which may trigger PMDD symptoms again in some women.[Cunningham J, et al]

Diuretics are used widely, under the assumption that many symptoms of PMS are secondary to fluid retention. Adverse effects include nausea, dizziness, palpitations, excess diuresis, and weakness.

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been used. Adverse effects include nausea, vomiting, epigastric pain, gastrointestinal (GI) bleeding, and rash.

NUTRITIONAL SUPPLEMENTATION AND HERBAL FORMULATIONS

Nutritional supplements often used by women in self-treatment of PMDD symptoms include the following:

- Vitamin B complex
- Calcium with magnesium chloride
- Evening primrose oil
- Kelp
- *L* - tyrosine
- Multivitamin-mineral complex with manganese
- Vitamin C with bioflavonoids

The use of pyridoxine (vitamin B-6) has had varying degrees of success, according to the literature. Calcium supplementation during the luteal phase has proven beneficial with regard to bloating, pain, mood, and food cravings. Administration of magnesium was helpful for premenstrual emotional and physical symptoms. Evening primrose oil contains the essential fatty acid gamma-linolenic acid and is sold widely as a nutritional supplement. Use of the oil is based on the premise that women with PMDD have a deficit of gamma-linolenic acid. Although clinicians believe the oil is of little value in treating PMDD, it is used widely as a nonprescription remedy for breast tenderness. [Thwe T Htay, MD]

DIET

Dietary advice constitutes an important aspect of nonpharmacologic treatment of PMDD. Reducing caffeine intake may minimize the potential adverse effects of excess caffeine consumption (eg, nervousness, jitteriness). Restricting sodium intake may reduce bloating. Some patients are able to avoid symptoms resembling hypoglycemia by reducing intake of highly refined carbohydrates and by having 5 or 6 smaller meals during the day instead of 3 large meals. [Thwe T Htay, MD]

ACTIVITY

Moderate aerobic exercise improved premenstrual symptoms. Traditionally, aerobic exercise is recommended, particularly if depressive or fluid retention symptoms predominate. From the available scientific data, it is unclear whether aerobic exercise is more effective than nonaerobic exercise. The efficacy of exercise could be the result of raised endorphin levels, physiologic changes, psychological changes, or combinations thereof. [Thwe T Htay, MD]

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Прогностично значение на del1p при пациенти с множествен миелом

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The prognostic significance of del1p in patients with multiple myeloma

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РЕЗЮМЕ:

Цел. Идентифициране на независими генетични прогностични фактори по отношение на преживяемостта при пациенти с множествен миелом (ММ) и значението им в ерата на новите терапевтични агенти. **Материал и методи.** При 92 новодиагностицирани болни с ММ са извършени флуоресцентна in situ хибридизация (FISH) и/или конвенционален цитогенетичен анализ. Използвани са специфични сонди за най-честите високорискови генетични маркери при ММ: del13, amp1q, del1p, del17p и t(4;14). За идентифициране на прогностични фактори по отношение на общата (OS) и свободната от прогресия (PFS) преживяемост е използвана регресия на Кокс. Анализът на преживяемостта е извършен с метода на Каплан-Майер и log-rank тест. Оценена е медианата на преживяемост в зависимост от наличието или липсата на определена високорисова аберация и вида индукционна терапия (бортезомиб-базирана или конвенционална). Статистическата обработка на данните е извършена с програма SPSS v21. **Резултати.** Независими прогностични фактори по отношение на PFS са само високите нива на β2-микроглобулин и del1p, докато високият β2-микроглобулин, тромбоцитопенията и del17p са независими предиктори за кратка OS. Сред генетичните маркери del17p и del1p имат най-голямо негативно влияние върху преживяемостта, независимо от вида на приложената индукционна терапия. Заключение. Пациентите с del1p следва да бъдат считани за високорискови, подобно на пациентите с del17p, и могат да бъдат кандидати за по-агресивна индукционна терапия.

Ключови думи: множествен миелом, , преживяемост, нови терапевтични агенти, del1p.

SUMMARY

Purpose. Identification of genetic markers as independent prognostic factors for survival in multiple myeloma patients and assessment of their significance in the era of novel agents. **Methods.** 92 newly-diagnosed multiple myeloma patients with performed FISH and/or conventional cytogenetics were evaluated. Specific probes were used for the most frequent high-risk genetic markers, including del13, amp1q, del1p, del17p and t(4;14). Prognostic factors for progression-free survival (PFS) and overall survival (OS) were identified by means of the Cox proportional hazard model for covariate analysis. Median survival times were calculated and compared according to the presence or absence of a particular high-risk aberration and the type of induction therapy (bortezomib-based or conventional). Kaplan-Meier curves were plotted and compared using the log-rank test. Statistical analyses were performed with the program SPSS v21. **Results.** In our analyses only high β2-microglobulin levels and del1p were independent prognostic factors for PFS, while high β2-microglobulin, thrombocytopenia and del17p were independent predictors of poor OS. Among the high-risk genetic aberrations del1p and del17p had the greatest negative influence on patients' outcome, regardless of the induction therapy performed. Conclusion. Patients with del1p should be considered high-risk, similarly to patients with del17p, and may be candidates for more aggressive induction therapy.

Key words: multiple myeloma, survival, novel agents, del1p.

INTRODUCTION

The genomic characteristics of the malignant clone are an important aspect of multiple myeloma pathogenesis. It is well known that the disease is associated with certain cytogenetic abnormalities, some of which confer poor prognosis. The detection of these abnormalities with fluorescence in situ hybridization (FISH) can identify a group of patients with high risk who should be treated differently compared to those with standard risk.

The purpose of this study is identification of genetic markers as independent prognostic factors for survival in multiple myeloma patients and assessment of their significance in the era of novel agents.

SUBJECTS AND METHODS

92 newly-diagnosed multiple myeloma patients with performed FISH and/or conventional cytogenetics were evaluated in this retrostpective study. Informed consent for genetic testing was obtained from all subjects. The median age of the patients was 63.6 (39-85) years. The rest of the patients' characteristics are summarized in **Table 1.**

FISH analysis was performed, using specific probes for the most frequent multiple myeloma high-risk genetic markers, including del13, amp1q, del1p, del17p and t(4;14): 13q14/13qter; 1p36/1q21, 17p13/SE17, 14q32 (BA), FGFR3/IGH. In cases conventional cytogenetics was used, at least 11 metaphases were analyzed and if a chromosomal rearrangement was detected – between 25 and 50 metaphases.

Prognostic factors for progression-free survival (PFS) and overall survival (OS) were identified by means of the Cox proportional hazard model for covariate analysis. As possible prognostic factors the following parameters were included in the regression model: age, β2-microglobulin levels, hemoglobin levels, platelet counts, creatinine, calcium, percent of bone-marrow infiltration, extramedulary disease, plasmablast morphology and presence or absence of the above mentioned genomic aberrations. Median survival times were calculated and compared according to the presence or absence of a particular high-risk aberration and the type of induction therapy (bortezomib-based or conventional). Conventional induction therapy included VAD regimen in transplant eligible patients and melphalan-based regimens in those who were ineligible for ASCT. The following bortezomib-based regimens used in transplant eligible patients : Vel/Dex - Bortezomib 1.3 mg/m^2 iv or sc D1, D4, D8, D11; Dexamethasone 40 iv D1-D2, D4-D5, D8-D9, D11-D12 and CyBorD: Bortezomib 1.3 mg/m^2 iv or sc D1, D4, D8, D11, Cyclophosphamide 300 mg/m^2 iv D1, D8, D15; Dexamethasone 40mg iv D1-D2, D4-D5, D8-D9, D11-D12. In transplant ineligible patients VMP regimen was used: Bortezomib 1.3 mg/m2 iv or sc D1, D4, D8, D11, D22, D25, D29, D32; Melphalan 9 mg/m^2 po D1-D4 and Prednisone 60 mg/m2 po D1-D4.

Statistical analyses were performed with the program SPSS v21. Kaplan-Meier curves were plotted and compared using the log-rank test.

RESULTS

The prevalence of high-risk genomic aberations in our patient cohort was as follows: del13 in 39 (42.4%) patients; amp1q - in 22 (23.9%); del1p and del17 each in 15 (16.3%), and t(4;14) in 8 (8.7%) patients.

A number of parameters were included in the regression model as possible prognostic factors in multiple myeloma patients: age, β2-microglobulin levels, hemoglobin levels, platelet counts, creatinine, calcium, percent of bone-marrow infiltration, extramedulary disease, plasmablast morphology, and presence or absence of t(4;14), del17p, del13, amp1q, del1p. In univariate analysis, several parameters were associated with shorter PFS **(Table 2).** In multivariate analysis only high β2-microglobulin levels (p=0,035) and del1p (p=0,000) were independent prognostic factors for PFS.

The parameters, associated with shorter OS in univariate analysis were: high β2-microglobulin, renal dysfunction, anemia, thrombocytopenia, plasmablast morphology, presence of del17p and del1p **(Table 3).** In multivariate analysis high β2-microglobulin (p=0.002), thrombocytopenia (p=0.037) and

Table 1. ***Patients' characteristics***

Characteristics	*Number (n=92)*	*Percent (%)*
1. Gender		
- male	49	53.3%
- female	43	46.7%
2. Myeloma type		
IgG	57	62%
IgA	17	18.5%
Light chain	18	19.6%
3. ISS stage		
I	12	13%,
II	27	29.3%
III	53	57.6%
5. Therapeutic approach		
- Eligible for ASCT *	46	50%
- Ineligible for ASCT	46	50%
- Bortezomib-based therapy	53	57.6%
- Conventional therapy	39	42.4%

** Patients were considered ASCT eligible if under 65 years of age and without significant comorbidity.*

Table 2. ***Parameters associated with PFS on univariate analysis***

Parameter (PFS)	p	HR	95% CI	
			Lower Bound	Upper Bound
β2-microglobulin (< 5,5/> 5,5 mg/L)	0,009	1,05	1,01	1,09
High creatinine levels	0,001	1,00	1,00	1,00
Low hemoglobin levels	0,009	0,98	0,96	0,99
Thrombocytopenia (< 120/> 120 G/L)	0,009	2,99	1,40	6,38
Plasmablast morphology (no/yes)	0,025	0,35	0,17	0,71
del1p (no/yes)	0,004	0,42	0,23	0,76

del17p (p=0.006) were independent predictors of poor OS.

Among the high-risk genetic aberrations del1p and del17p had the greatest negative influence on patients' outcome **(Table 4).** According to our results del17p and del1p were negative prognostic markers regardless of the induction therapy performed - conventional or bortezomib-based **(Table 5).** Patients with these two genomic aberrations had significantly shorter median PFS both after induction with conventional therapy and bortezomib. Median PFS after bortezomib-based induction was 9 months for patients with del1p vs. 26 months (p<0.001) for patients without del1p. In cases with and without del17p the median PFS was 9 months vs. 24 months (p=0.017), respectively. Patients with del1p and 17p had significantly shorter median survival, compared to patients without these aberrations, even after induction with a novel agent: 16 months vs. 79 months for del1p (p=0.014) and 12 month vs. 79 months for del17p (p=0.001) **(Fig. 1 and Fig. 2).**

DISCUSSION

In the studies by Marzin et al. (5) и Chang et al. (2,3) del1p21 was detected by FISH in 18-24% of the analyzed multiple myeloma patients. By using FISH and conventional ctogenetics in our patient cohort the percent of patients with del1p abnormality was close to that reported in the scientific literature – 16.3%. Deletions in region 1p21 were detected in 3 of 15 patients and del1p36 – in 6/15 patients. In the other 6 cases other regions of the short arm of chromosome 1 were deleted. It is suggested that del1p leads to hemyzygocy of at least one tumor suppressor gene. Such has been localized in the locus 1p36 (p73), which was the most frequently deleted in our patients. Still its role in the patogenesis of MM has not been confirmed (8).

Del17p is an adverse prognostic factor in MM which is included in the risk stratification models (4,6) and retains its significance even after treatment with novel agents (1). Presence of this aberration predicts short survival and such patients are candidates for clinical trials (6).

Del1p is another known negative prognostic factor in multiple myeloma. In their studies Chang et al. identify del1p as an independent risk factor for PFS (P = 0.01) and OS (P = 0.04 (2). Patients with 1p21 deletions treated with high-dose chemotherapy had significantly shorter progression-free survival (PFS; median 14.2 vs 25.4 months, P<0.001) and overall survival (OS; median 39.4 vs 82.3 months, P=0.001) than those without such deletions. In multivariate analysis, del1p21 was an independent poor prognostic factor associated with disease progression in MM (3). In another repot by Qazilbash et al. del1p was associated with a significantly shorter remission and survival in patients undergoing high-dose therapy and a single autologous transplant (7). However there are few data in the literature concerning the outcome of myelma patients with this abnormality, treated with bortezomib-based induction therapy.

According to our results del1p and del17p also appear to be the two strongest negative prognostic factors for PFS и OS. Bortezomib-based induction did not improve survival in patients with these cytogenetic abnormalities.

CONCLUSIONS

Regardless of the relatively small number of analyzed patients, our results lead us to the following conclusions:

- del1p is an independent negative prognostic factor for PFS and is associated with significantly shorter median survival in multiple myeloma patients.
- bortezomib - based therapy did not improve outcome in our patient cohort with del1p.
- patients with del1p should be considered high-risk, similarly to patients with del17p, and may be candidates for more aggressive induction therapy.

Table 3. ***Parameters associated with OS on univariate analysis***

Parameter (OS)	p	HR	95% CI	
			Lower Bound	Upper Bound
β2-microglobulin (< 5,5/> 5,5 mg/L)	0,008	0,43	0,23	0,80
High creatinine levels	0,001	1,00	1,00	1,00
Low hemoglobin levels	0,003	0,97	0,96	0,99
Thrombocytopenia (< 120/> 120 G/L)	0,000	5,77	2,58	12,90
Plasmablast morphology (no/yes)	0,004	0,35	0,17	0,72
del17p (no/yes)	0,000	0,31	0,16	0,59
del1p (no/yes)	0,001	0,32	0,15	0,64

Table 4. ***Survival of multiple myeloma patients according to the presence or absence of high-risk genetic aberrations***

Aberration	Median Survival (months)	95% CI		p	Median PFS (months)	95% CI		p
		Lower Bound	Upper Bound			Lower Bound	Upper Bound	
No del13	32,0	24,9	39,1	0,917	18,0	12,6	23,4	0,434
del13	43,0	28,4	57,6		14,0	5,1	22,9	
No del17p	43,0	30,3	55,7	<0,001	20,0	13,2	26,7	0,003
del17p	12,0	8,2	15,8		9,0	4,3	13,6	
No del1p	43,0	31,9	54,1	0,001	20,0	16,2	23,8	<0,001
del1p	12,0	0,0	28,2		8,0	2,5	13,5	
No amp1q	40,0	27,5	52,5	0,165	19,0	14,8	23,2	0,260
amp1q	32,0	19,3	44,7		11,0	8,4	13,6	
No t(4;14)	40,0	26,8	53,2	0,854	18,0	11,6	24,4	0,677
t(4;14)	20,0	11,9	28,1		10,0	7,9	12,0	
Overall	32,0	29,8	50,2		18,0	11,4	24,6	

Table 5. ***Survival of patients with and without del1p and del17p according to the induction therapy performed***

Therapy	Aberration	Median PFS (months)	95% CI			MS (months)	95% CI		p
			Lower Bound	Upper Bound			Lower Bound	Lower Bound	
Conventional	del1p	4,0	2,8	5,2	0,005	6,0	0,0	14,3	0,053
	No del1p	16,0	6,0	25,9		32,0	24,7	39,3	
Bortezomib	del1p	9,0	5,5	12,5	< 0,001	16,0	0,0	37,6	0,014
	No del1p	26,0	17,7	34,3		79,0	37,3	120,7	
Conventional	del17p	8,0	0,0	18,3	0,103	12,0	4,3	19,7	0,033
	No del17p	13,0	10,5	15,5		32,0	26,1	37,9	
Bortezomib	del17p	9,0	7,7	10,3	0,017	12,0	5,1	18,9	0,001
	No del17p	24,0	13,2	34,8		79,0	5,4	152,6	

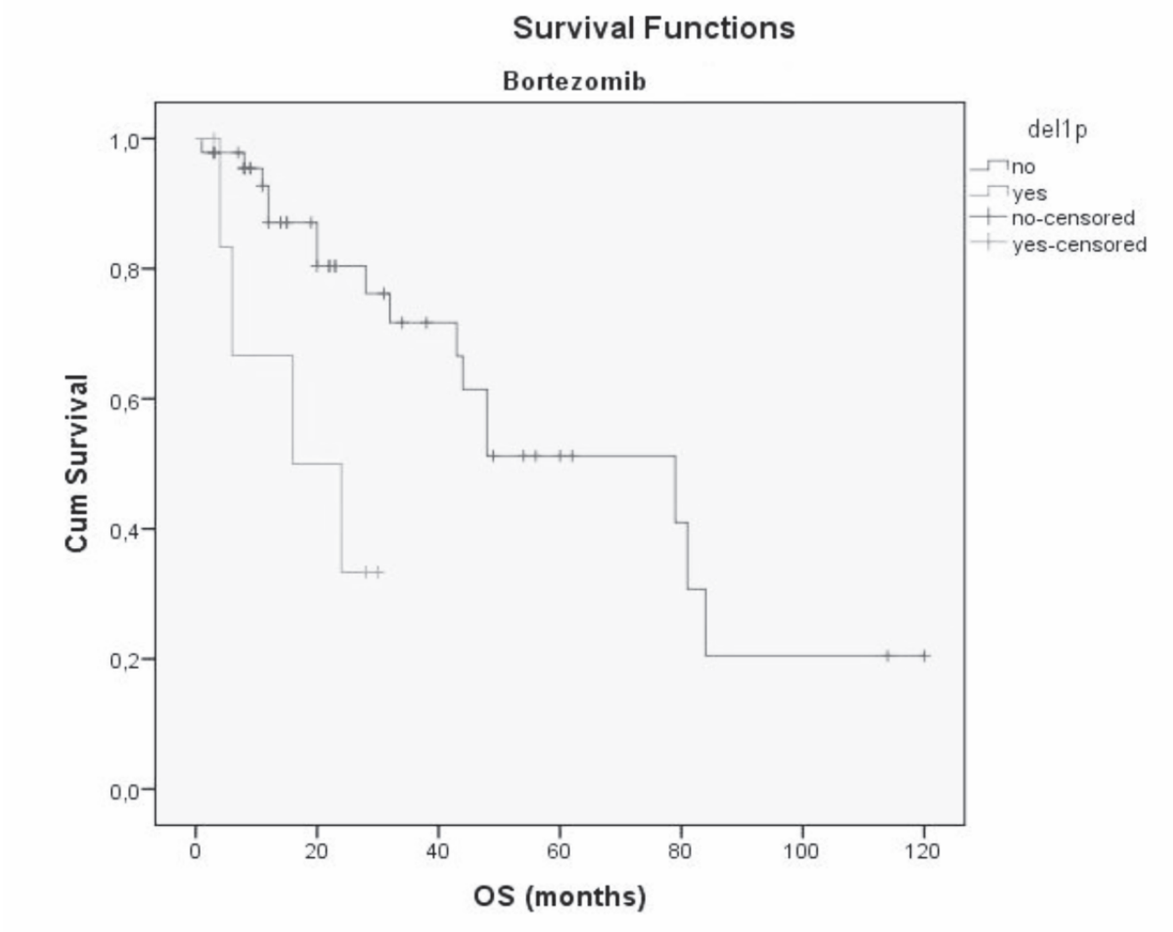

Fig. 1. ***OS according to the presence or absence of del1p in patients treated with bortezomib-based induction (p=0.014)***

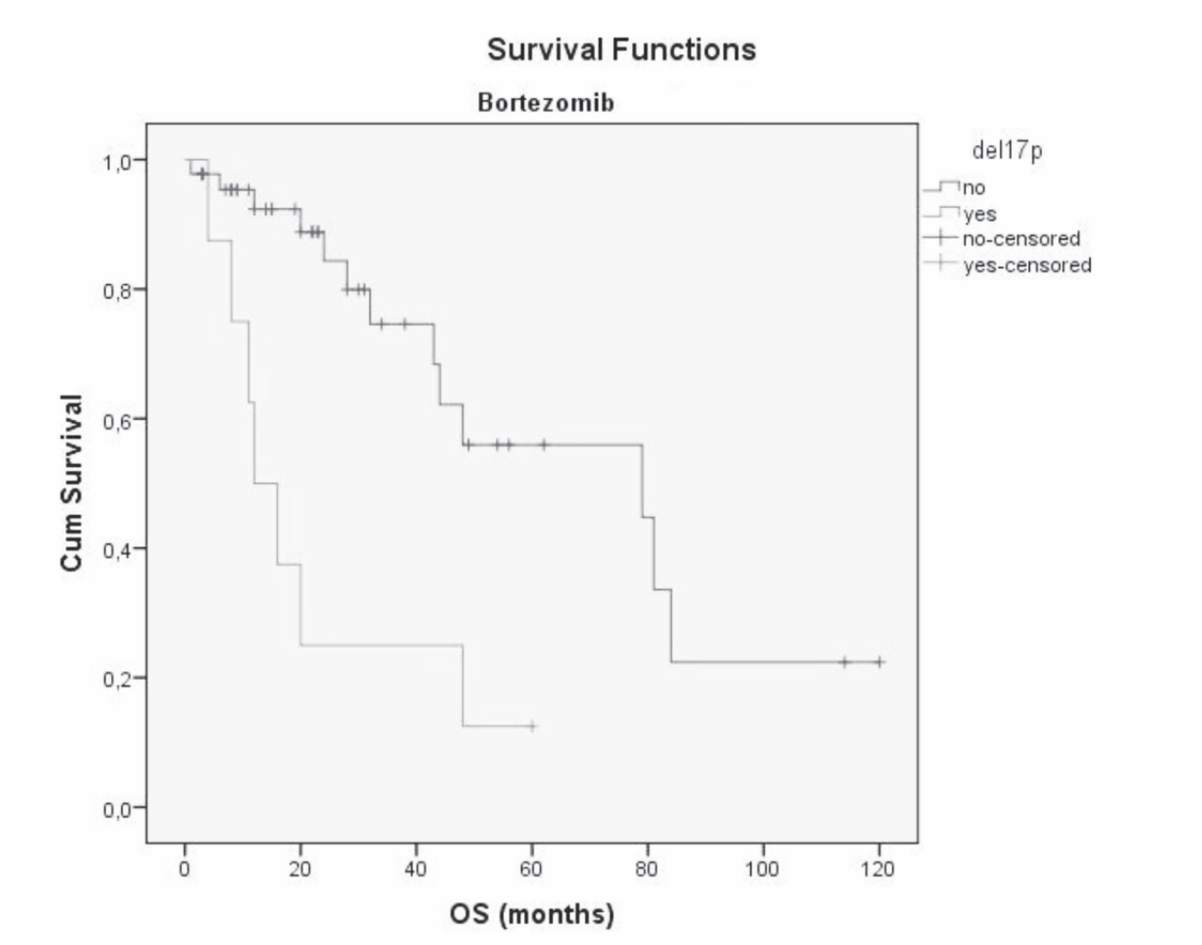

Fig. 2. ***OS according to the presence or absence of del17p in patients treated with bortezomib-based induction (p=0.001)***

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Експресия на CCR7, TACI и костимулаторните молекули CD40, CD80, CD86 и CD28 при хронична лимфоцитна левкемия

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Expression of CCR7, TACI and the costimulatory molecules CD40, CD80, CD86 and CD28 in chronic lymphocytic leukemia

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РЕЗЮМЕ:

Цел: Проучване на взаимовръзките между експресията на молекулите с отношение към клетъчната миграция и активация при хронична лимфоцитна левкемия (ХЛЛ).

Материал и методи: Изследвани са нивата на CD40, CD80, CD86, TACI, CCR7 върху B-клетките и CD28, CCR7 върху T-клетките при 98 нелекувани пациенти с ХЛЛ (стадирани според стадиращата система на Rai разпределени в 3 рискови групи).

ABSTRACT

Aim: To investigate relationships between molecules supporting cell migration and activation in chronic lymphocytic leukemia (CLL).

Materials and Methods: The levels of CD40, CD80, CD86, TACI, CCR7 on B-cells, and CD28, CCR7 on T-cells, was investigated in 98 untreated CLL-patients (staged according to Rai Staging System and stratified into 3 risk groups).

Results: The results showed increased levels of CD40 ($P < 0.001$), CD86 ($P = 0.029$) and CCR7 ($P < 0.001$); decreased expression

Резултати: Резултатите показват повишени нива на CD40 ($P < 0.001$), CD86 ($P = 0.029$) и CCR7 ($P < 0.001$); понижена експресия на CD80 ($P < 0.001$) и TACI ($P = 0.003$) върху В-клетките. Във всички рискови групи се установява положителна корелация между CD80 и CD86 ($P < 0.001$), както и между CD80 с CD40 ($P = 0.020$), TACI ($P = 0.024$) и CCR7+ В-клетки ($P = 0.015$). В общата група пациенти се установява връзка между CD28+ Т-клетки и CCR7+ Т-клетки ($P = 0.002$; rho = + 0.401), в частност в групата с нисък ($P < 0.001$; rho = + 0.688) и интермедиерен риск ($P < 0.001$; rho = + 0.712). Експресия на CD80 над 20% се установява при 17 пациента, като при тях корелацията между CD80 и TACI се усилва ($P < 0.001$; rho = + 0.785). Значимо повишение на тази връзка се намира също и при пациенти с TACI над 60% (n = 22; P = 0.004; rho = +0.602).

Заключение: Експресионните нива на В-клетъчните костимулаторни молекули включени в проучването, показват повишена активация и дефицит на de novo експресия на CD80 при болшинството пациенти. Наличието на ХЛЛ-пациенти с активиран антигенен профил, предполага различен клиничен ход на заболяването.

Ключови думи: хронична лимфоцитна левкемия; CCR7; TACI; CD80; CD86; CD28

Conclusion: Expression levels of B-cell costimulatory molecules, included in this study, show increased activation and deficient de novo expression of CD80 in the majority of patients. The presence of CLL-patients with an activated antigen profile, suggests different clinical course of the disease.

Keywords: chronic lymphocitic leukemia; CCR7; TACI; CD80; CD86; CD28

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a disease with high variability in its clinical manifestation and course. It was considered an indolent, antigen inexperienced disease of slowly accumulating cells, but researchers now accept that CLL-cells are proliferative [12], antigen experienced cells. The survival, and proliferation of CLL-cells is mediated by exposure to chemokines, cytokines, antigenic stimulation and intercellular contacts [10]. Chemokines CCL19 and CCL21 regulate the recruitment of lymphocytes into the T-cell zone areas of the secondary lymphoid tissues through ligation to their corresponding receptor CCR7 [15]. CLL-cells express high levels of CCR7 (CD197) [16, 3], which are higher in patients with prominent lymphadenopathy [16, 19]. T-cells are important for B-cell activation and proliferation [6, 1]. The basic molecules for interaction between the B- and T-cells are the costimulatory molecules CD80 and CD86, expressed on the B-cells. CD80 and CD86, which are attributed to B7-molecules, play different roles in immune modulation depending on the ligand to which they connect themselves - CD28 or CD152, on the surface of the T-cell. The connection with CD28 conducts costimulatory signals leading to activation, differentiation and an effective cellular and humoral immune response. The connection with CD80 with CD152 has a stronger affinity than the connection of CD86 with CD152, while CD28 connects CD86 more effectively

than CD80. The cells at rest express lower levels of CD86 [9]. Unlike normal B-lymphocytes, CLL-cells are weak antigen-presenting cells due to the reduced expression of costimulatory molecules which leads to a defect in the formation of immunological synapse with the T-cells [10, 5]. CD267 (TACI - Transmembrane Activator Calcium modulator and cyclophilin ligand Interactor) is receptor of BAFF (B-cell-Activating Factor) and APRIL (A Proliferation-Inducing Ligand), contributing to the differentiation and survival of B-cells). CLL-patients exhibited variable TACI expression, with the majority of cases displaying low TACI. CLL-cells with high TACI expression displayed a better survival capacity in vitro, when cultured with BAFF and/or APRIL [11]. The aim of the present study is to examine expression levels of the chemokine receptor CCR7 on the B- and T-lymphocytes in parallel with the expression of TACI, the costimulatory molecules CD40, CD80, CD86 on the B-cells and the T-cell expression of ligand CD28, and also to establish their correlations in patients with CLL and in the low, intermediate and high risk subgroups.

MATERIALS AND METHODS

Patients

Ninety-eight previously untreated patients with CLL (54 men and 44 women) were included in the study. The median age of patients was 66.7 years, ranging from 37 to 84 years.The patients were staged according to Rai Staging System and divided into 3 subgroups: low (Rai 0), intermediate (Rai I / II) and high risk (Rai III / IV) **(Table 1).** The control group of 17 age-matched healthy volunteers was also included, consisting of 11 male and 6 female subjects with a median age of 63 years (range: 39–78 years). Complete

Table 1. ***Clinical features of the CLL-patients (n = 98)***

Gender (M% / F%)	**54** (55.1%) / **44** (44.9%)				
Age years (median P10 - P90)	**66.7** (52.2 – 78.4)				
Stage to Rai staging system	**Rai 0**	**Rai I**	**Rai II**	**Rai III**	**Rai IV**
Patients number (%)	31 (31.63)	18 (18.37)	18 (18.37)	15 (15.31)	16 (16.33)
Distribution by risk	**Rai 0**		**Rai I / II**		**Rai III / IV**
Patients number (%)	31 (31.63))		36 (36.73)		31 (31.63)
WBC [G/l]	**34.49** (14.2 – 167.9)				
Ly [G/l]	**27.1** (8.6 – 143.44)				
CD19+ [G/l]	**21.33** (5.6 – 129.4)				
HGB x [g/dl]	**13.9** (10.2 – 15.6)				
PLT [G/l]	**200.5** (101.3 – 295.2)				
sTK1 [U/l]	**2.8** (0.0 – 74.9)				

The values are presented as median (P10 – P90), WBC – leukocytes, Ly – lymphocytes, HGB - haemoglobin, PLT – platelets, sTK1 – serum thymidine kinase-1.

blood count, detection of levels on serum thymidine kinase-1 (sTK-1) (Thymidine kinase REA KIT; Beckman Coulter) **(Table 1)** and immunophenotyping were performed in all cases. Diagnosis was based on the criteria of WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Fourth Edition [13]. The study was approved by the local ethics committee and written informed consent was obtained from all subjects, in accordance with the Declaration of Helsinki.

Immunophenotyping, antibodies and analysis

Flow cytometric analysis was performed on peripheral blood in K2EDTA, diluted to approximately 1 x 10^6 lymphocytes/ml with FACS Wash Buffer (BD Biosciences, San Jose, CA, USA). For each tube 50 □l from the sample were incubated with 5□□l of each fluorochrome-conjugated monoclonal antibody for 30 minutes in the dark at room temperature (20° to 25°C), followed by 10 minutes lysing with FACS Lysing Solution (BD Biosciences, San Jose, CA, USA) and washing. Cell samples were analyzed by six-colour flow cytometry (FACSCanto II, BD Biosciences, San Jose, CA, USA) using the FACSDiva 6.0 software (BD). At least 50000 events were acquired. The diagnostic phenotyping panel of monoclonal antibodies included: CD19, CD20, CD22, CD79b, CD5, CD23, FMC-7, CD200, CD3, CD4, CD8, CD16+56, □ and □ light chains (BD Biosciences, San Jose, CA, USA). The panel investigating activation receptors included: CD28 PerCP-Cy5.5; Clone: L293 (BD Biosciences), C40 APC; Clone: 5C3 (eBioscience), CD80 PE-Cy7; Clone: L307.4 (BD Pharmingen), CD86 PerCP-Cy5.5; Clone: 2331 (FUN-1) (BD Pharmingen), CD197 FITC; Clone: 150503 (BD Pharmingen), CD267 PE; Clone: 1A1-K21-M22 (BD Pharmingen). The flow cytometry results were presented as follows: CD19+ cells were presented as a percentage of lymphocytes, as well as an absolute count; in regard to other antigens percentages of positive cells out of CD19+ B-cells and/or out of CD3+ T-cells, were recorded.

Statistical analysis.

Statistical analysis was carried out using the SPSS 21.0 software package (SPSS Inc., Chicago, Illinois, USA). Continuous data parameters were analyzed for normality using the W-Shapiro–Wilk test; data were presented as median and (P10 – P90), because most of the variables had nonsymetric (non-Gaussian) distribution. Nonparametric tests of U-Mann–Whitney and Kruskal–Wallis (for continuous variables) were used. Spearman's rank correlation (rho) was used to measure the relationship between variables. Groups were assumed to differ significantly when the *P* value was less than 0.05 and highly significant when the *P* value was less than 0.001.

RESULTS

Median (P10 - P90) expression levels of the activation markers included in the study are listed in Table 2. The levels of all studied membrane receptors on B-cells of patients with CLL show statistically significant differences compared to healthy subjects. The expression of CD40 ($P < 0.001$), CD86 ($P = 0.029$) and CD197 ($P < 0,001$) is significantly increased, while the levels of CD80 ($P < 0.001$) and CD267 ($P = 0.003$) are decreased. No significant differences in the expression of CD28 and CD197 on T-lymphocytes were found between leukaemia patient samples and the control group. In the comparison of the levels of all studied receptors, no significant differences were found between the three risk groups **(Table 2)**.

The chemokine receptor CD197 expressed from malignant cells showed positive correlations with the total count of WBC ($P < 0.001$), lymphocytes ($P < 0.001$) and monoclonal B-cells ($P < 0.001$) in the general group of patients with CLL, and in all three subgroups **(Table 3).**

The receptors included in the study show correlations of variable strength between themselves in the general group of patients with CLL **(Table 4).**

Expression levels of CD40 showed positive correlation with the levels of CD80 ($P = 0.020$) and CD197 ($P = 0.032$) expressing B-cells. The correlations of CD80 with CD86 ($P < 0.001$), CD267 ($P = 0.024$) and CD197+ B-cells ($P = 0.015$) are positive. The percentage of the T-cells expressing CD28 showed direct correlation with the levels of the chemokine

Table 2. ***Comparisons of expression levels of parameters on B- and T-lymphocytes and P value***

	Controls (n = 17)	CLL (n = 98)	*P* value	Rai 0 (n = 31)	Rai I / II (n = 36)	Rai III / IV (n = 31)	*P* Value
B-cells							
CD40+ [%]	**95,1** (86.0 – 99.3)	**99.7** (92.0 – 99.9)	< **0.001**	**99,5** (91.7 – 99.9)	**99,6** (92.9 – 99.9)	**99,8** (82.9 – 100)	**NS**
CD80+ [%]	**27.3** (17.9 – 43.4)	**6.65** (0.72 – 49.8)	< **0.001**	**7.3** (0.6 – 37.7)	**8.6** (1.5– 61.0)	**5.1** (0.4 – 56.4)	**NS**
CD86+ [%]	**9.5** (2.3 – 19.0)	**18.8** (2.5 – 54.1)	**0.029**	**19.5** (2.4– 55.7)	**20.8** (2.4 – 56.2)	**15.5** (1.9 – 47.2)	**NS**
CD197+ [%]	**29.6** (1.2 – 60.5)	**99.5** (92.1 – 99.9)	< **0.001**	**99.1** (89.0 – 99.8)	**99.5** (91.2 – 99.8)	**99.6** (86.4 – 100)	**NS**
CD267+ [%]	**41.6** (27.1 – 59.9)	**12.0** (0.75 – 81.2)	**0.003**	**5.3** (0.4 – 77.0)	**11.6** (1.6 – 81.6)	**24.9** (0.0 – 74.0)	**NS**
T-cells							
CD28+ [%]	**69.0** (42.3 – 88.0)	**60.4** (25.3 – 86.1)	**NS**	**67.7** (24.7 – 89.2)	**60.4** (41.1 – 83.2)	**58.9** (20.1 – 86.8)	**NS**
CD197+ [%]	**57.0** (23.6 – 66.9)	**46.2** (24.4 – 70.2)	**NS**	**48.5** (27.7 – 72.2)	**41.9** (25.0 – 63.5)	**48.3** (15.9 – 79.3)	**NS**

Median (P10 – P90)

receptor CD197 on T-cells (P = 0.002) and inverse correlation with its expression on B-lymphocytes (P = 0.043). The established correlation between the two costimulatory molecules CD80 and CD86 in malignant population was observed in all three risk subgroups (Rai 0: P = 0.032; Rai I / II: P = 0.011; Rai III / IV: P = 0.002), while the correlation between the CD80 and CD267 positive B-cells was observed only in low risk group patients (P = 0.010). The CD86+ B-cells correlate with the levels of CD267+ B-cells (P = 0.042) and with concentration of the proliferation marker sTK1 (P = 0.018) in the intermediate risk group. The correlation between the chemokine receptor CD197 on T-cells with CD28+ T-cells was observed in the patients from the low and intermediate risk groups (Rai 0: P < 0.001; Rai I / II: P < 0.001) **(Table 5)**.

Despite the strongly decreased median expression of CD80 and CD267 on the monoclonal B-cell population, in some of the patients the two receptors have increased levels compared to the levels observed in healthy subjects. Expression of CD80 higher than 20% was found in 17 patients, equally distributed within the risk subgroups (low risk: n = 6; intermediate risk: n = 5; high risk: n = 6), with the correlative dependency between CD80 and CD267 increasing significantly in these subjects (P < 0.001; rho = +0.785). Expression of CD267 higher than 60% was found in 22 patients, with 50% of them being in the Rai IV stage (low risk: n = 6; interme-

diate risk: n = 5; high risk: n = 11), with the correlative dependency between CD80 and CD267 being significantly increased in these patients too (P = 0.004; rho = +0.602).

DISCUSSION

Interaction of monoclonal B-cells with T-cells is a stimulus for activation and proliferation [6]. In patients with CLL, T-cells are a significant fraction of lymphoid infiltration located around and within proliferation centres [14]. The chemokine receptor CCR7, whose main role is lymphocyte homing to secondary lymphoid tissues, shows significantly increased levels on malignant B-lymphocytes compared to the control group, while the expression on T-cells is without statistically significant differences which suggests that T-cell migration in CLL is not amplified by this mechanism. The correlations between expression of CD197 on the B- and T-lymphocytes with expression of CD28 as well as between the expression of CD197 and CD40 on the B-cells probably reflect the influence of CCR7 on costimulatory signals conducted through the pathways CD40 – CD40L and CD80/CD86 – CD28, increasing cell migration and homing. After the activation of the B-cell receptor, CD40 is a key regulator of the B- and T-cell interactions, it is stimulated by T-cells expressing CD40L [8], located together with CLL-cells in the proliferation centres in secondary lymphoid tissues. The receptor CD40, constitutionally expressed by B-cells, is registered in increased levels on monoclonal lymphocytes. CLL-cells in cases of in vitro activation CD40 enter the cell cycle [4] and avoid apoptosis [7, 17]. The CD40 - CD40L connection leads to activation of Nuclear factor – □B (NF□B), and increased expression of the costimulatory molecules CD80 and CD86 on B-lymphocytes. The connection of CD80 / CD86 with CD28 expressed by T-cells is a costimulatory signal leading to activation, differentiation and an effective cellular and humoral immune response. The resting cells express lower levels of CD86, activation through CD40 leads to increased expression of CD86 and *de novo* expression of CD80 [9]. The established phenotypic profile of the studied receptors in peripheral blood shows increased activation of leukemic cells. Increased levels of CD40 and the receptor CD86, connecting CD28 more effectively than CD80 [9], as well as the positive correlation between CD80 and CD86 are evidence pointing to that. Lower expression of CD80 on B-cells found in this study suggests impaired signalling pathways in the leukemic cells leading to decreased capacity of de novo expression of CD80 in a large portion of the patients. In the cases of patients with increased expression levels the signalling pathway for activation of CD80 is probably preserved or compensated by the increased signal of the CD40 - CD40L connection. The

Table 3. ***Correlations between CD197+ B-cells [%] and leukocytes***

	CLL (n = 98)	Rai 0 (n = 31)	Rai I / II (n = 36)	Rai III / IV (n = 31)
WBC [G/l]	$P < 0.001$ (rho□□□+ 0.452)	$P = 0.003$ (rho□□□□+ 0.515)	$P = 0.039$ (rho□□□□+ 0.346)	$P = 0.003$ (rho□□□□+ 0.537)
Ly [G/l]	$P < 0.001$ (rho□□□□+ 0.462)	$P = 0.003$ (rho□□□□+ 0.515)	$P = 0.020$ (rho□□□□+ 0.385)	$P = 0.003$ (rho□□□□+ 0.547)
CD19+ [G/l]	$P < 0.001$ (rho□□□□+ 0.478)	$P = 0.002$ (rho□□□□+ 0.530)	$P = 0.016$ (rho□□□□+ 0.400)	$P = 0.002$ (rho□□□□+ 0.552)

Table 4. ***Correlations between laboratory parameters on B- and T-cells in CLL patients (n = 98)***

	CD80+ B-cells [%]	CD86+ B-cells [%]	CD197+ B-cells [%]	CD267+ B-cells [%]	CD28+ T-cells [%]	CD197+ T-cells [%]
CD40+ B-cells [%]	$P = 0.020$ (rho□□□□+ 0.311)	NS	$P = 0.032$ (rho□□□□+ 0.277)	NS	NS	NS
CD80+ B-cells [%]		$P < 0.001$ (rho□□□□+ 0.513)	$P = 0.015$ (rho□□□□+ 0.313)	$P = 0.024$ (rho□□□□+ 0.217)	NS	NS
CD197+ B-cells [%]				NS	$P = 0.043$ (rho□□□□- 0.272)	NS
CD28+ T-cells [%]						$P = 0.002$ (rho□□□□+ 0.401)

Table 5. ***Correlations between investigated molecules on B- and T-cells in CLL risk groups***

Parameter	Rai 0	Rai I / II	Rai III / IV
CD80+ B-cells [%]	**CD86+:** $P = 0.032$ (rho□□□□+ 0.415) **CD267+:** $P = 0.010$ (rho□□□□+ 0.488)	**CD86+:** $P = 0.011$ (rho□□□□+ 0.446)	**CD86+:** $P = 0.002$ (rho□□□□+ 0.568)
CD86+ B-cells [%]	-	**CD267+:** $P = 0.042$ (rho□□□□+ 0.356) **sTK1:** $P = 0.018$ (rho□□□□+ 0.428)	-
CD28+ T-cells [%]	**CD197+ T-cells:** $P < 0.001$ (rho□□□□+ 0.688)	**CD197+ T-cells** $P < 0.001$ (rho□□□□+ 0.712)	-

established correlation between the expressions of CD40 and CD80 gives a reason for this hypothesis. The mechanisms of the B- and T-cell dysfunction are not completely understood. The expression of the studied surface active molecules and observed correlations in the risk subgroups suggest various degrees of deregulation in the two main lymphocytic populations over the course of the disease. The costimulating receptor CD86 shows correlation with sTK1 registering proliferation activity and TACI in the intermediate risk group of patients, probably due to activation under the influence of the factors acting in the specific microenvironment. Studies on the levels of TACI on CLL-cells have established the significantly lower expression registered in this study too, and also the presence of inverse correlation between the levels of this molecule, the Rai stage and leukocyte count [11, 2]. The present study did not find correlations between the TACI levels and adverse clinical factors. The correlation between CD267 and CD80 is strong when there is increased expression of CD80 or CD267. TACI connects with two ligands – BAFF and APRIL, which transmit stimuli which play a major role in the survival of normal B-cells. CLL-cells with high expression of TACI in co-culture with BAFF and/or APRIL show a better survival capacity [11], moreover, the BAFF – TACI connection stimulates B10 activity in patients with progressive CLL and healthy subjects, while in the context of malignant disease, IL-10 production is a significant contributor to immunosuppression. [18]

CONCLUSION

The observed levels of the costimulatory receptors CD40, CD80, CD86 in peripheral blood show increased activation and probably impaired signals for de novo expression of CD80 in the majority of CLL-patients, while the correlations in high expression levels of TACI and CD80 suggest the presence of a group of patients with an activated antigen profile and probably a different clinical course of the disease.

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Ефект на фрагмента на аргинин вазопресина (4-9) върху пространственото ориентиране на плъхове с лезии на префронталната кора

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Effect of arginine-vasopressin fragment (AVP 4-9) on spatial orientation in rats with a lesion of the prefrontal cortex

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РЕЗЮМЕ:

Аргинин-вазопресинът и неговият фрагмент (AVP 4-9) участват в реализирането на някои мозъчни функции. Те упражняват специфичните си ефекти преди всичко върху процесите на заучаване и запаметяване. Цел на това проучване е изследване ефектите на фрагмента AVP (4-9) върху пространственото ориентиране във воден лабиринт на плъхове с лезии на префронталната мозъчна кора. Шест групи животни бяха използвани – 3 с фалшиви лезии и 3 с лезии. Прилагани са 2 дози на фрагмента AVP (4-9) . Получените резултати показват, че фалшиво оперираните животни заучават задачата и я запаметяват. Плъховете с фалшиви лезии и третирани с AVP (4-9) показват подобряващ ефект върху обучението и паметта. Плъховете с

ABSTRACT

Arginine-vasopressin fragment (AVP 4-9) is involved in some brain functions. It exerts its specific effects on avoidance behavior as well. The aim was to study the effects of AVP (4-9) on spatial orientation in rats with lesion of prefrontal cortex in Morris water maze. Six groups of animals were used: 3 with sham lesion and 3 with real lesion. Two doses of AVP (4-9) were used respectively. The obtained results shows, that the sham rats learned the task during learning session and keep it on memory retention test. Rats with sham lesion, treated with AVP (4-9) in two doses showed the improving effect of AVP (4-9) on learning and memory tests. The animals with lesion in prefrontal cortex had impaired learning and memory. AVP (4-9) improved the spatial orientation in rats with lesion. All this data per-

лезии на префронталната мозъчна кора показват увредена способност за заучаване и запаметяване. Животните с лезии, третирани с AVP (4-9) също показват подобряващ ефект върху обучението и запаметяването, макар и по-слабо изразен. Получените данни позволяват заключението, че префронталната мозъчна кора е структура, важна за реализиране на процесите на обучение и запаметяване. AVP или неговият фрагмент AVP (4-9) играят ключова роля в този процес. Ефикасността на вазопресиновите фрагменти може да бъде полезна при третиране в клинични условия на когнитивни дефицити, дължащи се на повлияване на области, тясно свързани с хипокампалната функция.

Ключови думи: фрагмент на аргинин-вазопресин, воден лабиринт на Морис, лезии на префронтална кора

mitted the conclusion, that pre-frontal cortex is the brain structure important for learning and memory functions and AVP or its fragment AVP (4-9) play a pivotal role in it. The efficacy of vasopressin like fragments may especially be useful in individuals with cognitive deficits due to those areas which are closely associated with hippocampal functioning.

Key words: Arginine-vasopressin fragment, Morris water maze, prefrontal cortex lesion

INTRODUCTION

Arginine-vasopressin (AVP) is a non-peptide hormone which mediates both peripheral and central functions. Several studies, mainly performed with rats in aversively motivated tasks suggest that AVP and related peptides affect learning and memory processes (7). It is assumed that vasopressin in the brain is broken down into shorter fragments which exert their specific behavioral effects as evidence by marked influence on avoidance behavior (13). In addition, AVP (4-9) fragment reduced the latency to press a lever to obtain food and decreased the number of trials to attain maximum performance, particularly when the conditioned stimulus was of long duration. Also in a number of studies in patients with cognitive impairments (12, 17) which beneficial effects on short-term memory for words, numbers and spatial orientation of arginine-vasopressin have been shown. It is assumed that the nature and the location of the cognitive impairment in combination with the tasks used to determine the way vasopressin influences the performance are important.

The psychopathology of human studies which reported positively on vasopressin effects indicated that the prefrontal cortex is involved. The ventromedial prefrontal cortex is part of two parallel limbo-thalamic pathways pivotal for memory and recognition (1).

Prefrontal cortex (PFC) lesions in rats made a deficits in temporal ordering of events, expressed in cognitive behavioral tasks as delayed response, delayed alteration and delayed matching to sample tasks and spatial orientation in the Morris maze (2). Besides these common behaviors, species-typical behaviors, which require a high degree of temporal organization, are also known to be affected as a result of PFC damaged (18).

Broersen et al (3) found that the lesions of the median prefrontal cortex (mPFC) disturb performance in a variety of delay tasks, which suggests that the mPFC supports short-term memory processes. Functions of ventro-median prefrontal cortex are closely associated with those ascribed to the hippocampus and amygdale which is probably due to connections between those structures and the infra- and prelimbic area (11).

Therefore, in the present study the efficacy of a vasopressin fragment AVP(4-9) was studied by lesioning the pre- and infra-limbic area which resulted in an impaired performance in a spatial orientation task.

MATERIAL AND METHODS

1. Animals

Forty five male Wistar rats (TNO, Zeist, The Netherlands), weighing 190-210 g at the time of lesion the prefrontal pre/infralimbic area (PFC IL and PL) were used. The animals were randomly divided into 6 groups. The animals were housed in groups of 2-3 in Macrolon cages at temperature (21 ±1° C) and light-controlled room with reversed day/night cycle (red light was switched on at 08.00 h and switched off at 20.00 h). Food and tap water were available ad libitum. The groups were as follows:

S0 – sham lesion (N = 6) treated subcutaneously (s. c.) with saline in a volume of 0.1ml/100g body weight. For all behavioral tasks the animals received a single injection prior to every block of trials in case of the Morris maze prior to every observation of the other tasks.

S1 – sham lesion animals were treated 30 min before each observation with AVP (4-9) with a dose of 0.1mg/kg sc, N = 8.

S2 – sham lesion animals were treated before each observation with AVP (4-9) with a dose of 0.01mg/kg sc, N = 7.

L0 – lesion animals were treated with saline (in the same volume and same manner as the experimental group), N = 7.

L1 – lesion animals were treated 30 min before each observation with AVP (4-9) at a dose of 0.1mg/kg sc, N = 8.

L2 – lesion animals were treated 30 min before each observation with AVP (4-9) at a dose of 0.01 mg/kg N = 8.

To avoid any stress due to the transportation or any other changes in environmental circumstances all behavioral experiments were carried out in a room adjacent to the experimental set up at least 24 hours prior to the experiment and took place between 10.00 and 15.00 h.

2. Surgery

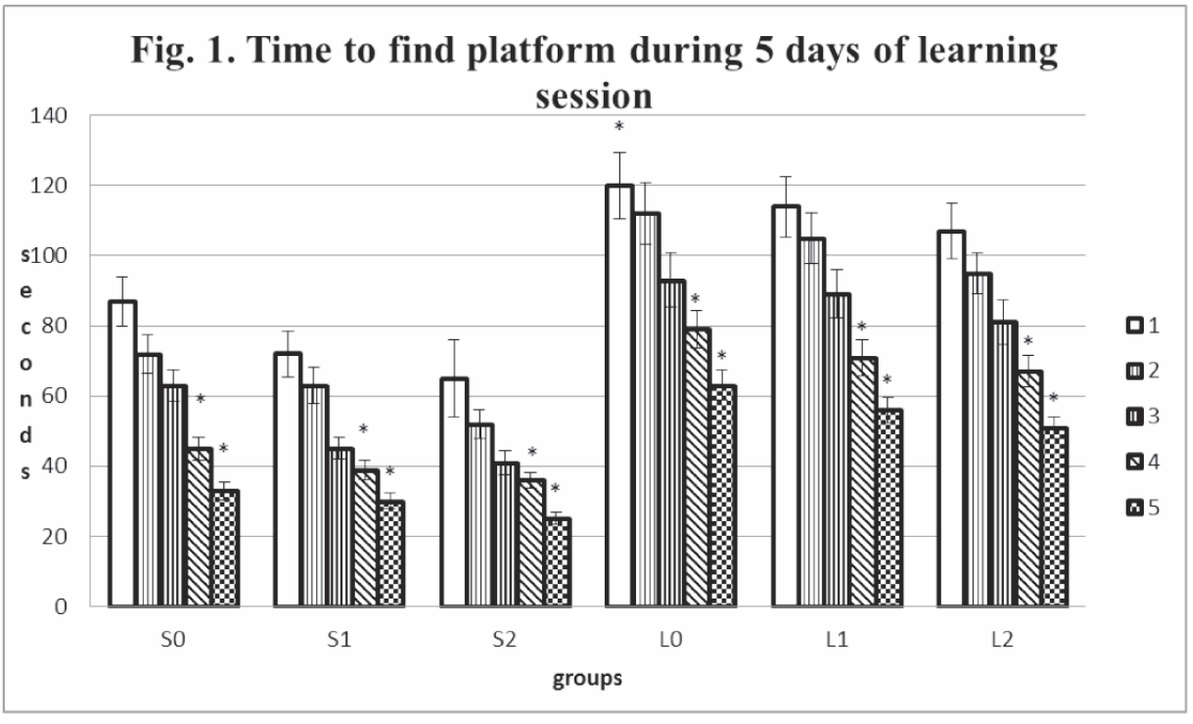
The rats were anaesthetized with a subcutaneous injection of Hypnorm (0.05 ml/100g) (Duphar, Weesp, The Netherlands) containing flunisone (10mg/ml) and fentanyl citrate (0.2 mg/ml). After placement in the stereotactor the skull was exposed and small holes were drilled bilaterally for the placement of the silver electrodes. Through a current of 0.2 mA for a duration of 20 sec using a Radio Frequency Lesion Maker (Phillips, The Netherlands) lesions were made. After lesion the animals were allowed to recover for 2 weeks. The experiments started when they had reached the weight of the day of the surgery (after approximately 2 weeks).

Corrected for the difference in body weight the location corresponds with coordinates 3.20 mm posterior from bregma according to the atlas of Paxinos and Watson. We used the following coordinates A3.2, L 0.6 and D 0.5. The sham lesion animals received the electrodes at the same position but without any current.

3. Behavioral testing

Behavioral testing began 15 days after surgery. Rats were first tested in the water maze for 5 days and 7 days later was performed memory task.

Morris maze task was performed in a circular pool, 210 cm in diameter and 50 cm deep. The tank was filled with warm water (25 cm) of approximately 26° C. A plastic black platform (8 cm diameter) was placed in the pool; 1 cm below the surface of the water rendered the platform invisible to the rats. Behavioral tests were performed under dim red light conditions. The pool was located in a large observation room, which external cues outside the pool. These cues were kept unchanged throughout the period of testing. A trial started by placing a rat into the water facing the wall of the pool at one of 4 starting positions, which divided the pool into 4 quadrants of equal size. The platform was located in a constant position in the middle of quadrant 2, equidistant from the centre and the end of the pool. The animals received a block of 4 trials with an inter-trial interval of 15 min on 5 consecutive days with different sequence of starting points on every day. If the rat did not find the platform within 120s, it was placed on it at the end of the trial and remained there for 30 s, subsequently a score of 120 s was given. On the day 5, after the last block of acquisition trials, a single trial was carried out: the rat was allowed to swim for 60 s in the pool without platform to assess the searching strategy of the animals. Swimming pattern were registered by computerized image analysis system (Ethovision, Noldus B.V. Wageningen, as described before by us).



4. Histology

At the end of the observation the rats were injected intraperitoneally with overdose of pentobarbital (Euthesate, Apharma). Brains were removed after decapitation in 4% formalin containing glasses. Thionine stained sections of 50 μm with 500 μm between the sections were used for histological examination of the location of the lesion with thionine staining.

5. Statistics

The data have been analyzed with a two way ANOVA with repeated measurements for evaluating the effect of AVP (4-9) on the Morris maze. The mean values and the standard errors of the means were calculated.

RESULTS

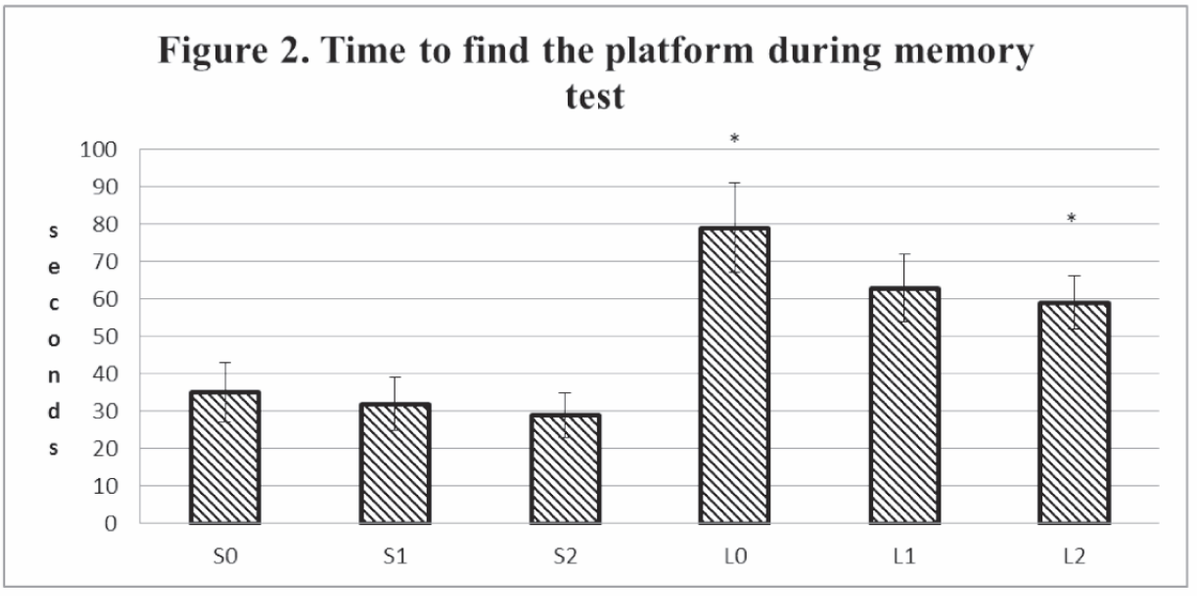
The rats from the sham control (SO) group decreased the time spend to find the platform statistically significant on 4th and 5th days of learning session ($P<0.05$) compared to the 1st day **(Fig. 1).** The sham groups (S1 and S2) treated with AVP (4-9) also learned the task and decreased the time spend to find the platform on 4th and 5th days of learning session, compared to the respective 1st day ($P<0.05$), as well as during all days of training ($P<0.05$) compared to the respective days of SO group **(Fig. 1).**

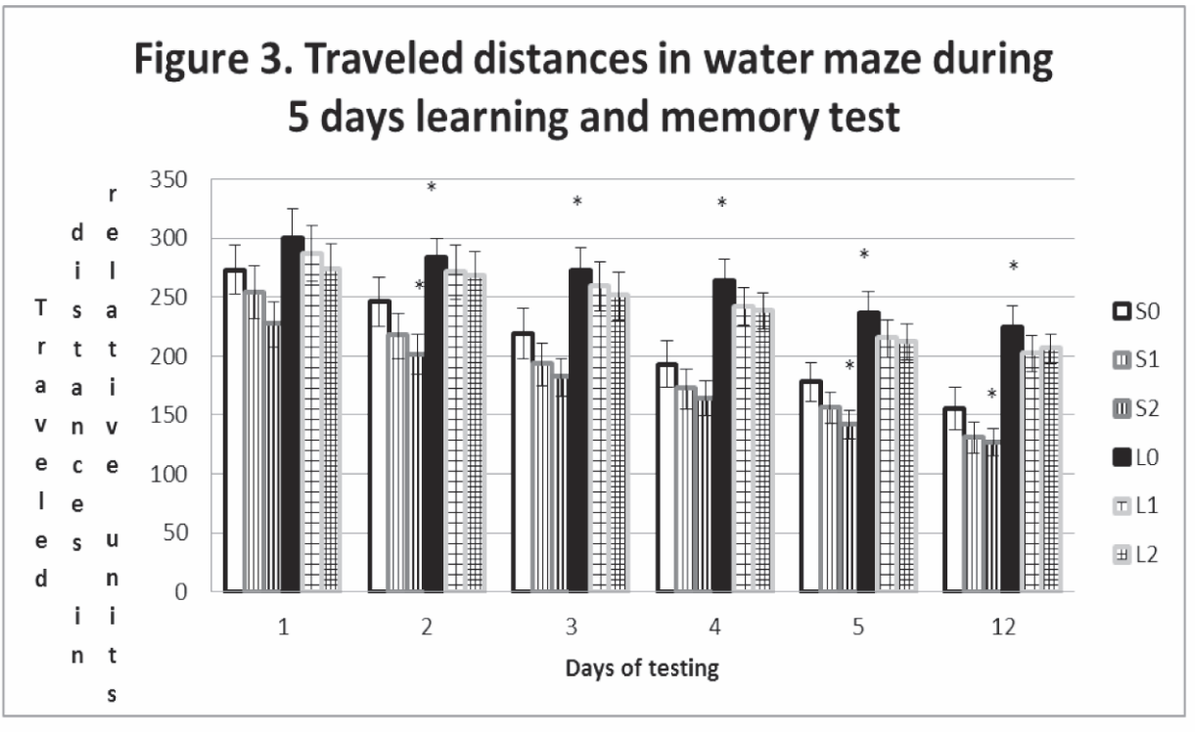
The rats with lesion of pre-frontal cortex (LO) group increased the time spend to find the platform statistically significant on all days of learning session ($P<0.05$) compared to the respective days of SO group **(Fig. 1).** The rats with lesion of pre-frontal cortex (L1 and L2) and treated with AVP (4-9) decreased the time spend to find the platform statistically significant ($P<0.05$) on all days of learning session ($P<0.05$) compared to the respective days of LO group, as well as during all days of learning session compared to the 1st days of training of the same group **(Fig. 1)**.

The rats from the sham control (SO) group do not change the time spend to find the platform, compared to the last (5th) day of learning session **(Fig. 2).** The same effect was observed for the rats (S1 and S2) treated with AVP (4-9) compared to the last (5th day) of learning session of the same groups **(Fig. 2)**.

The rats with lesion of pre-frontal cortex (LO) group do not change the time spend to find the platform, compared to the last (5th) day of learning session **(Fig. 2)**. The same effect was observed for the rats with pre-frontal cortex lesion (L1 and L2) treated with AVP (4-9) compared to the last (5th day) of learning session of the same groups **(Fig. 2).**

The rats from the sham control (SO) group do not show statistically significant differences





in the traveled distances during first 2 days of learning, but decreased it on 3rd, 4th and 5th days of learning session, as well as on memory retention test **(Fig.3).** The sham groups (S1 and S2) treated with AVP (4-9) also decreased the traveled distances on 4th and 5th days of learning session and on memory test, compared to the respective 1st day ($P<0.05$), as well as during all days of training ($P<0.05$) compared to the respective days of SO group **(Fig. 3).**

The rats with lesion of pre-frontal cortex (LO) group statistically significant increased the traveled distance during learning session, as well as on memory retention test , compared to the respective days of SO group **(Fig.3).** The groups (L1 and L2) with pre-frontal cortex lesion, treated with AVP (4-9) also increased the traveled distances on all learning session and on memory test, compared to the respective days of SO group. The same groups (L1 and L2) with pre-frontal cortex lesion, treated with AVP (4-9) decreased the traveled distance significantly ($P<0.05$) during learning and on

memory test, compared to the respective 1st day of learning session **(Fig. 3).**

DISCUSSION

Our results permitted the suggestion, that the lesion of pre-frontal cortex of rats impaired learning and memory processes, due to increased time to find the platform and increased traveled distance during learning session, as well as during memory test. The lesion which primarily concerned the pre- and the infra-limbic area affect the conditioned avoidance behavior (5). The question arises whether this difference can be specifically ascribed to an improvement of the lesion-induced impairment in cognitive abilities.

The most striking result was the impairment in the transfer trial as seen in the lesion animals. The effect of the lesion appears mainly limited to the spatial orientation in the Morris maze. The impairment in spatial orientation is in agreement with the forwarded connection with the septo-hippocampal system (20).

Treatment with AVP (4-9) of rats with sham lesion showed considerable improvement of rat's performance during both learning and memory retention sessions. In our experiments AVP (4-9) had appeared to restore the lesion-induced deficit in spatial orientation as well showing improvement effect as well.

Arginine-vasopressin has been associated with stress responses of the cardiovascular, neuro-endocrine and behavioral activity (4, 19). The improvement seen in our results with the Morris maze performance is to be ascribed to indirect influences on an alteration in stress responses (9, 10). In agreement with the number of previous studies the peptide has demonstrated specific cognitive enhancing abilities. The efficacy of vasopressin on consolidation and retrieval has been primarily assessed in conditioned avoidance tasks and, through less extensive, in social recognition (151). The effect in non-aversive (food rewarded, sexually motivated etc) tasks is more controversial (6, 8). In a study on the efficacy of AVP (4-9) on medial frontal cortex animals with lesion (6) no effect was found. The differences in binding between AVP and AVP (4-9) according to them led to the conclusion that AVP (4-9) is more effective on changing arousal (increased non-selective attention) rather than cognitive enhancing. In addition, binding places are not necessarily receptors and as forwarded above, local injections of the fragment into septum-hippocampus were also effective (14).

Therefore, we are of the opinion that the receptor of AVP (4-9) is still to be characterized and the efficacy of the fragment on cognition depends on the combination of the substrate involved on the lesion site and the behavior task used. Brain structures such as ventral hippocampus and septum are associated with spatial orientation and the injection AVP (4-9) in those areas has resulted in behavioral efficacy. The memory effect might be explained by enhancing excitation of limbic areas as shown by a vasopressin increased response to glutamate, normalized LTP (16). The AVP (4-9) facilitation of spatial orientation in the animals with lesion may appear, because the intact animal perform also very well, which explain their improving effect.

Thus, the efficacy of vasopressin like fragments may especially be useful in individuals with cognitive deficits due to those areas which are closely associated with hippocampal functioning and an improved performance may rely on a facilitation of hippocampal functioning.

Disclosure: Authors declare no potential conflict of interest.

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Необичайно бързо злокачествено развитие/дедиференциация при солитарен фиброзен тумор: Описание на случай

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Unusual Rapide Malignant Progression/Dedifferentiation in Solitary Fibrous Tumor: A case report

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РЕЗЮМЕ:

Солитарният Фиброзен Тумор (СФТ) на плеврата съставлява 1-2% от първичните тумори с тази локализация. Отличава се с бавен растеж и рядко рецидивиране , което го класифицира като нискостепенен тумор с бенигнено или гранично поведение. Злокачественият СФТ се среща много по-рядко.Заема около 10% от всички плеврални СФТ. Той може да възникне de novo или чрез дедиференциация, развила се в нискостепенни СФТ. Дедиференцираните варианти обикновено се появят в рецидиви след много години, но не винаги може да се предвиди малигнената еволюция на тумора. Дедиференциацията се характеризира най-често от рязък преход между добре диференцирания компонент на туморните и нискодиференцираните зони и е асоциирана с по-агресивно биологично поведе-

ABSTRACT

Solitary fibrous tumor (SFT) of the pleura constitutes 1-2% of primary tumors with this localization. It is characterized by slow growth and rarely recurrense, and thus is classified as a low-grade tumor with benign or borderline behavior. Malignant SFT is much more rare - about 10% of all pleural SFTs. It can arise de novo or by dedifferentiation occurring in a low-grade SFT. Dedifferentiated SFTs usually appear in recurrences after many years, but we cannot always predict malignant tumor evolution. Dedifferentiation is characterized by a sharp transition between well-differentiated components of the tumor and high-grade areas and is associated with aggressive biological behavior. Only 8 cases of Dedifferentiated SFTs were published in the available literature, without details about the terms of their development after the first operation. We describe a case with unusual

ние. В литературата са публикувани само 8 случаи на дедиференцирани СФТ на плеврата, но не са отбелязани сроковете на развитието им след първата операция. Ние описваме случай на необичайно бързо развитие на дедиференциация на солитарен фиброзен тумор на плеврата. Това е и първото публикувано описание на този вид тумор в България. Биопсии на 71-годишна жена, бяха изпратени в нашата клиника за второ мнение и имунохистохимична верификация, след като са били извършени две гръдни операции. Първоначалната диагноза след първата операция е била СФТ на плеврата. След втората операция, направена 10 месеца по-късно, се диагностицира злокачествен вариант на този тумор. Ревизията на биопсиите при нас потвърждава диагнозите при двете операции. Диагнозата се основава на морфологичната картина при двата варианта с изявената агресивна еволюция при втората биопсия, бърз растеж, проявен с висока пролиферативна активност, цитологичен атипизъм и инфилтративни прояви към белодробния паренхим.

rapide development of unpredictable dedifferentiation of a solitary fibrous tumor of the pleura. To the best of our knowledge his is the first published case of this type of tumor in Bulgaria. Biopsies of 71-year-old woman were sent to our clinic for a second opinion and immunohistochemical verification after two thoracic operations were performed. The initial diagnosis after the first operation was pleural SFT. After the second operation, carried out 10 months later, the diagnosis was malignant SFT. The revision of biopsies confirmed the initial diagnosis in both operations. Diagnosis is based on the typical morphological picture in the two specimens with overt aggressive evolution in the second biopsy, rapid growth proved by high proliferative activity, cytological atypism and infiltrative growth in the lung parenchyma.

INTRODUCTION

Solitary Fibrous Tumor (SFT) is an uncommon (1-2%) borderline tumor (previously referred to as localized fibrous tumor) often discovered as an asymptomatic lesion on routine chest radiographs in patients of any age, with no sex predilection, and with no evident relation to asbestos exposure. Most SFTs arise at the level of the visceral pleura. Although they may grossly appear to infiltrate the pulmonary parenchyma, they usually have a sharply delimited pushing border [4,12,14].

Grossly the lesion is well circumscribed, firm, lobulated, gray-white to yellow-white, with frequent whorling and fasciculation. The mean diameter is 6cm. The gross appearance is reminiscent of uterine leiomyoma. Microscopically, there is a tangled network of fibroblast-like cells, squeezed in between abundant collagen fibers, many of which have a keloid-like quality. The degree of cellularity and polymorphism varies a great deal from area to area. Haemangio pericytoma-like areas are frequent. Once accepted as distinct tumor now hemangiopericytoma considered to represent a cellular variant of SFT (1). Some tumors have prominent myxoid features. Nuclear pleomorphism is absent, mitoses are rare or none [10].

Malignant SFTs are rarer than benign SFTs. England et al.(3) Langman(7) propose high cellularity, mitotic activity (4 or more mitoses per 10HPF), pleomorphism, haemorrhage, necrosis and cystic degeneration as key features of malignant SFT.

Immunohistochemically benign and malignant SFTs can be distinguished also with stains for CD34, p53, p16 etc. [4,10,12,14]. **(Tabl.1).**

CASE REPORT

Biopsies of a 71 year old woman (D.N.M. BN15223-15227/15) were sent to our department for a second opinion after an inferior right pulmonar lobectomy was performed and 10 months later followed by a right pulmonectomy.

Table 1. ***The most frequent monoclonal antibodies used in the diagnosis of SFT***

	Benign SFT		*Malignant SFT*	
	Literature data	*Our result-1biopsy*	*Literature data*	*Our result-2 biopsy*
CD34	+	+	- (±)	+
p53	- (±)	-	+	+
p16	- (±)	no examined	+	no examined
CD99	+	no examined	±	no examined
S100	-	-	-	-
Desmin	-	-	-	-
EMA	±	-	±	-
AE1/AE3	-	-	-	-
BCL2	+	+	+ (±)	+
Calretinin	-	-	-	-

The initial diagnosis after the first operation was SFT. After the second operation the diagnosis was malignant SFT. The histological re-evaluation was made on the two biopsied slides.

The first biopsy demonstrated low cellularity with prominent collagen fibres, without mitotic figures and necrosis. The spindled cells predominantly monomorphic were arranged haphazardly or in short fascicles. **(fig.1)** Immunohistochemically, the tumor cells were positive for CD34 **(fig.2)** and Bcl2, negative for Calretinin, EMA and CD99 ; Ki67 shows positive nuclear expression in 10% of tumor cells.

The second biopsy showed significantly changed microscopically features with increased signes of malignancy. Areas of low differentiated tumor were observed adjacent to well differentiated zones **(fig.3).** These areas were comprised of spindle cells, with marked hypercellularity, high mitotic activity, cellular and nuclear atypia, necrotic zones **(fig.4).** Tumor infiltrated lung tissue **(fig.5).**

Immunohistochemical results were analogical with these of the first biopsy with exclusion of Ki-67 reaction **(tabl.1).** The tumor had a high proliferative index-Ki67 positive nuclear staining of over 70% of the tumor cells **(fig.6).**

The final diagnosis allowed the confirmation of the initial diagnosis- SFT with malignization /dedifferentiation developped soon after the first operation.

DISCUSSION

The dedifferentiated SFT is an extremely rare neoplasm. In a large series study by Mosquera et al [8], spanning the course of 20 years (from 1988 to 2008) only 8 cases out of 948 SFTs were dedifferentiated SFTs (0,84%). Analysing the 8 reported cases, Mosquera et al showed that the age of the patients was 40-76 years old. All these cases had clear morphological signs of atypia and high mitotic indexes (some up to 25 mitoses per 10HPF). Dedifferentiation is a phenomenon, which is well described in soft tissue and bone tumors [6]. It may arise de novo (combined with well-differentiated tumor) or develop in a recurrence of a prior well-differentiated malignancy. Morphologically, dedifferentiation is characterized most often by abrupt transition between the well-differentiated component of the tumor and high-grade areas, and confers more aggressive biologic behaviour [5,6,8,13].

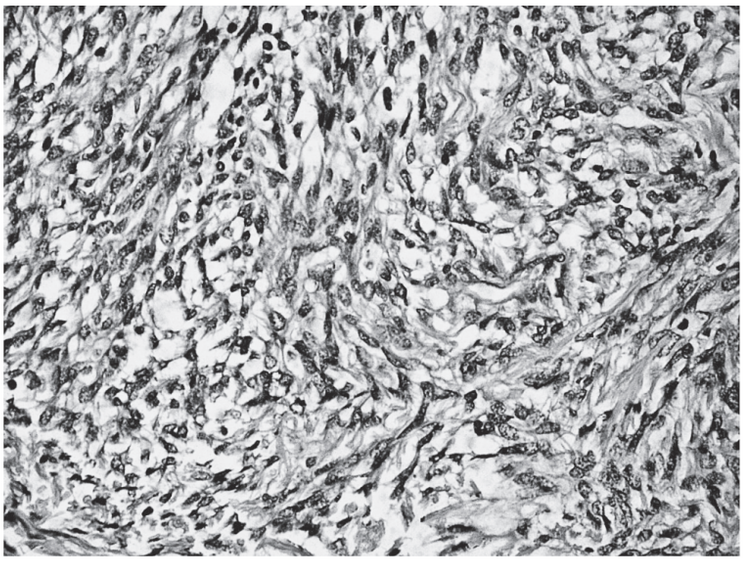

Figure 1. ***Well differentiated SFT – collagen fibers, fibroblast-like cells forme fascicles with a lot of collagen fibres among tumor cells. (First biopsy) H&E staining***

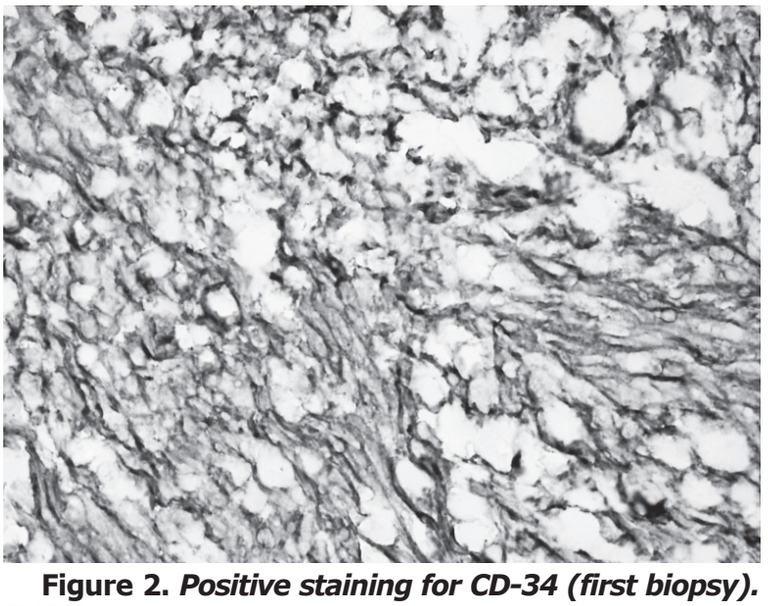

Figure 2. ***Positive staining for CD-34 (first biopsy). IHC staining***

Several histopathological criterias have been reported to be useful for determining malignancy in SFT. These include increased tumor size, mitotic count, cellularity, presence of necrosis/haemorrhage, nuclear pleomorphism /3,5,9/ and presence of sharply demarcated anaplastic/dedeifferentiated foci./1/. The study of Demicco et al created a risk stratification model based on age, size and mitotic index clearly delineated patients at high risk for poor outcomes.While small tumors with low mitotic rates are highly unlikely to metastazise, large tumors above 15 cm,which occur in patients above 55 years with mitotic figures above 4/10 high-power fields require close follow-up and have a high risk of both metastasis and death.

The biology of SFTs is somewhat unpredictable, and there is no assurance that a „benign" SFT will halt to a well-differentiated state and not progress to a „dedifferentiated" version of itself (although this occurrence is very rare – less than 1% of all SFTs) [5,13,8,6]. A few cases were publicated in which a dedifferentiation could be predicted by a presence of foci of histological malignancy observed in first biopsy. But common opinion exist that the clinical behavior of individual tumors is notoriously difficult to predict.(3,9)

Figure 3. ***Abrupt difference between well- and low differentiated areas in the second biopsy. Increased cellularity and histological atypism are evident. H&E staining***

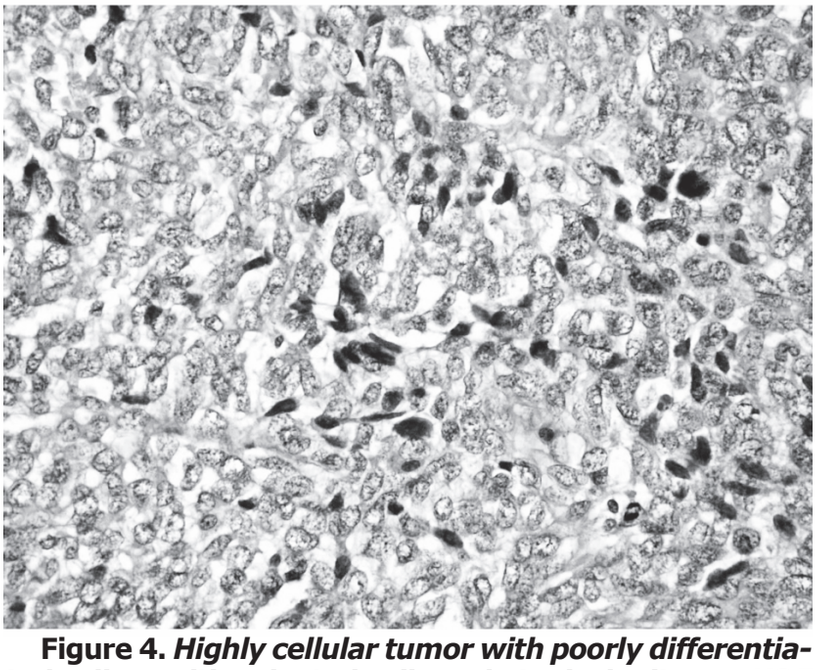

Figure 4. ***Highly cellular tumor with poorly differentiated cells, multinucleated cells and atypical mitoses. H&E staining***

The tumor cellularity or pleomorphism are not prognostic factors.They can be significant only in poorly differentiated areas which occurs as an undifferentiated high grade anaplastic sarcoma sharply demarcated from areas of conventional SFT. (1)

The immunohistochemical results in our case were the basis of making a differential diagnosis with other tumors such as mesothelioma, synovial sarcoma, thymoma, MPNST. We have no possibility to apply STAT6 staining specific for this tumor, which has been recommended recently for the differential diagnosis of pleural tumors [2,13,15]. The most frequently immunohistochenical markers using for the diagnosis of SFT are these showing differentiation in the cells. CD34 is a surface glycoprotein, functions as adhesion molecule , and facilitate migration of haemopoetic cells. Its presence in the non-haemopoetic cells has been linked to progenitor

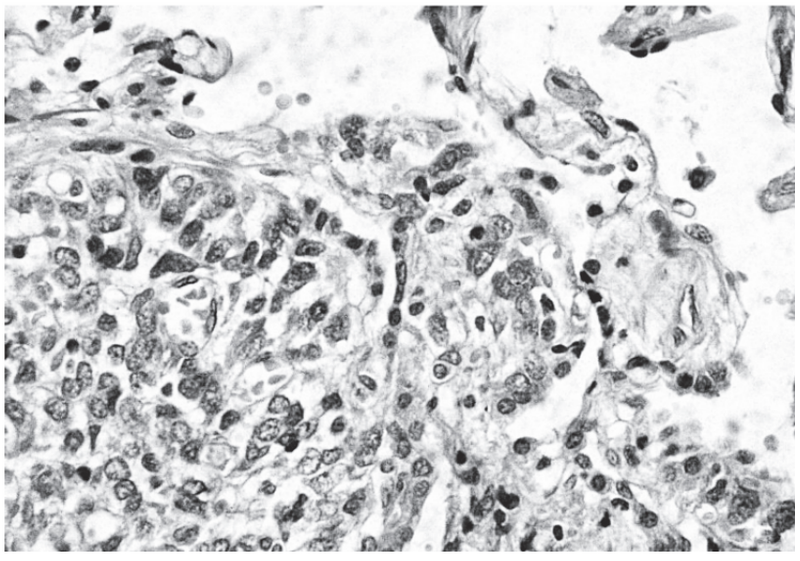

Figure 5. ***Infiltrative growth of the malignant SFT into the pulmonary parenchyma. H&E staining***

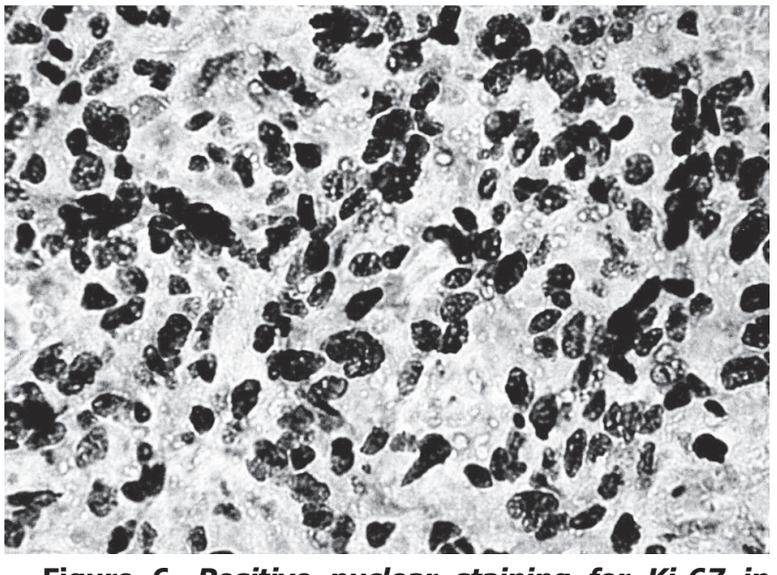

Figure 6. ***Positive nuclear staining for Ki-67 in most of tumor cells. IHC staining***

and adults stem cell phenotype (11). CD 34 is the most suitable marker for diagnosis of SFT although it could be expressed in other soft tissue tumors as dermatofibrosarcoma protuberans, and gastrointestinal tumors. Cytokeratins,EMA, desmin,SMA, S-100, calretinin were applied to discriminate SFT from epithelial, synovial, mesothelial and other mesenhymal tumors.Some markers as Bcl2,p16,CD99 and p53 show some nuances in its expression in benignes and malignant variants of SFT.

The typical features of malignant SFT in the high-grade component of the tumor, along with classic SFT morphology in the low-grade component, and significant difference in morphological feature in two biopsies support the diagnosis Dedifferentiated SFT rather than immunohistochemical data.The abrupt increase of proliferative index (Ki-67 posistivity) in the second biopsy correlated with aggressive behaviour in our patient and in publicated analogic cases.

To the best of our knowledge this is the first published case of a dedifferentiated SFT in Bulgaria. We acknowledge that the term „dedifferentiation" is controversial (6) and not that well defined but we believe that the case we described herein, falls within the currently accepted parameters for this concept.

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Хемоперитонеум, дължащ се на гастроинтестинален стромален тумор в тънките черва - клиничен случай

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Hemoperitoneum due to gastrointestinal stromal toumors of the small intestine - a case report

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РЕЗЮМЕ:

Гастроинтестиналните стромални тумори (ГИСТ) са редки тумори – 1-3% от всички гастроинтестинални злокачествени образувания. Кървене в перитонеалната кухина дължащо се на ГИСТ на тънкото черво се докладва в 1.4% от случаите. Имахме шанса да оперираме по спешност пациент с хемоперитонеум дължащ се на ГИСТ на тънкото черво. Туморите на тънкото черво често се оперират без точна предоперативна диагноза. Така е и в случая, който оперирахме. Прави се преглед на литературата.

Ключови думи: гастроинтестинален стромален тумор, пемоперитонеум.

SUMMARY

Gastrointestinal stromal tumors (GISTs) are rare tumors – 1-3 % of all gastrointestinal malignancies. Intra-abdominal bleeding due to GIST of the small intestine is reported in 1.4% of cases. We had the chance to treat on emergency base a patient with hemoperitoneum due to GIST of the small intestine. Tumors of the small bowel are often operated on without correct diagnose. This is true for the case we operated. A review of the literature is done.

Key words: gastrointestinal stromal tumor, hemoperitoneum.

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are rare tumors – 1-3 % of all gastrointestinal malignancies. They are the most common gastric and small bowel mesenchymal tumors (14). The length and relative inaccessibility of the small bowel have long constrained the diagnosis. Similar is the reason for the no correct diagnose reported by Zbigniew et al (18) – they publish a paper about 44 women with GIST of the small intestine 16 of which were operated in gynecological departments due to the tentative diagnosis of gynecological neoplasm. The opposite situation is in the case of Colombo et al (4) which localized intra-abdominal fibromatosis of the small bowel, who was diagnosed as gastrointestinal stromal tumor.

Rarer the tumor may have extra gastointestinal localization. Goh et al (6) report 8 out of 156 patients. Two of them were in the greater omentum, two in the lesser sac, lesser omentum, retro peritoneum, small bowel mesentery and pancreas. Extra gastro-intestinal GISTs (EGIST) are significantly larger than extramural or intra/trans mural GISTs. Most cases demonstrate some form of communication or contact with the gut wall.

Han et al (8) retrospectively analyze 141 patients with primary malignant tumor of the small bowel and find that the most common initial clinical features are intermittent abdominal discomfort or vague abdominal pain (67.4%), abdominal mass (31.2%), bowel obstruction (24.1%), hemotochezia (21.3%), jaundice (16.3%), fever (14.2%), coexistence of bowel perforation and peritonitis (5.7%), coexistence of gastrointestinal bleeding and shock (5.0%) and intra-abdominal bleeding (1.4%). The same authors report that the tumor is most often found in the ileum (44.7%), followed by jejunum (30.5%) and duodenum (24.8%).

Matek J. and Krska Z. (12) discuss intussusception on the small bowel the lead point of which was GIST. Being one of the rarest causes of ileus it was proved by abdominal CT. Trifan et al (16) report 5 small bowel tumors out of 102 in which capsule endoscopy was done. Kovacs et al (11) use not only capsule endoscopy but also double-balloon enteroscopy to diagnose small bowel tumors including GIST. Chen et al (3) perform 440 double-balloon endoscopy (DBE) examinations in 400 patients. Small bowel tumor were diagnosed in 78 of whom 67 were diagnosed using DBE (16.8% - 67/400); the other 11 patients had negative DBE findings and were diagnosed through surgery or capsule endoscopy. They compered CT with DBE and concluded that DBE had higher positive detection rate (67/78 – 85.9%) than CT (51/70 – 72.9%). De Siol et al (15) – a retrospective study evaluated 114 patients who underwent ultrasound-guided biopsy of gastrointestinal masses. Of 114 lesions they evaluated 112 were malignant (98.2%) and 2 benign (1.8%). Specimens were obtained from the stomach (38 – 33.3%), small bowel (36 – 31.6%), and colon (40 – 35.1%). According to their data diagnose was correct in 113/114 cases (99.1%). The only complication they report is 1 (0.9%) – bleeding from a gastric GIST.

Some of these tumors were miss diagnosed as leiomyosarsoma (9). Tumor size, mitotic count and site of origin are the three key prognostic factors, with the mitotic count being the singe strongest predictor of recurrence. Tumors arising in the small bowel have worse prognosis than those of comparable size and mitotic count arising in other organs (7). Wu et al (17) on the base of 100 patients with GIST of the small intestine conclude that tumors with low cellularity, low mitotic count and low Ki-67 index predict more favorable disease free survival. According to the same authors absence of tumor perforation with low mitotic count and low cellularity can predict long term overall survival.

Bay et al (2) think that more attention should be paid to the male patients with small intestine stromal tumors, especially those with tumor size > 5 cm. because those tumors are more likely to metastasize than smaller tumors (< or = 5 cm.). According to the data of Agaimy A. and Wunsch PH. (1) regional node metastasis were found in 2 out of 210 GISTs (1%). Patients were < or = 40 years. This fact suggests the need for node sampling in this particular group of patients although the prognostic significance of nodal metastases remains to be further clarified. This opinion is in controversy with the case reported by

El Demellawy et al (5) – a 79 year old female with GIST of the small intestine which metastasized to the regional mesenteric lymph nodes at the time of primary surgery.

Joensuu (10) proposes patients with certain no gastric tumors and those with tumor rupture to be included in the NIH (National Institute of Health consensus classification system) high risk category. Surgery (open or laparoscopic) remains the only curative option, but recurrence rates are high. Adjuvant therapy with ,fmfclonal antibody preparations as imantinib mesylate improves recurrence-free survival rates and may improve overall survival. For patients with advanced disease, first-line imatinib and second-line sunitinib malate have improved progression-free and overall survival rates. It would be ideal if the amount of these drugs could be adjusted according to each patient because they have various side effects and are very expensive (13).

CASE REPORT

KPK, 54 years old male was admitted in the hospital on the 27. IV. 2014 with pain in his abdomen without vomiting. On examination his abdomen was distended, painful when palpated more on the right side of his abdomen with not voluntary rigidity of the rectus muscles and lateral abdominal muscles, more distinct on the right side, rebound tenderness. Routine laboratory test were not helpful to diagnose. Abdominal ultrasound was misinterpreted as enlarged gall bladder. Technical reasons did not allow us to perform a CT which would have been very beneficial. An emergency operation was done. We found a tumor on his small intestine localized on the jejunum about 50 cm distally of the Treitz ligament, 12 cm large infiltrating the bladder and about 500 cc blood and clots.

Partial resection of the small intestine and the bladder were done and the abdomen was thoroughly cleaned and closed. Postoperative period was uneventful. Light microscopy Б-14г № 186, 187, 188, 189 /12. V. 2014 proved that it was GIST of the small intestine. Immunohistochemistry N: И – 189/14 /13. VI. 2014 confirmed that diagnose – gastrointestinal stromal tumor of the jejunum with low mitotic index (1 mitosis to 50 hpf). Prognostic group was defined as 3b. No tumor was found on the resected part of the bladder. Two months postoperatively PET-CT was done – there was no tumor anywhere in his body.

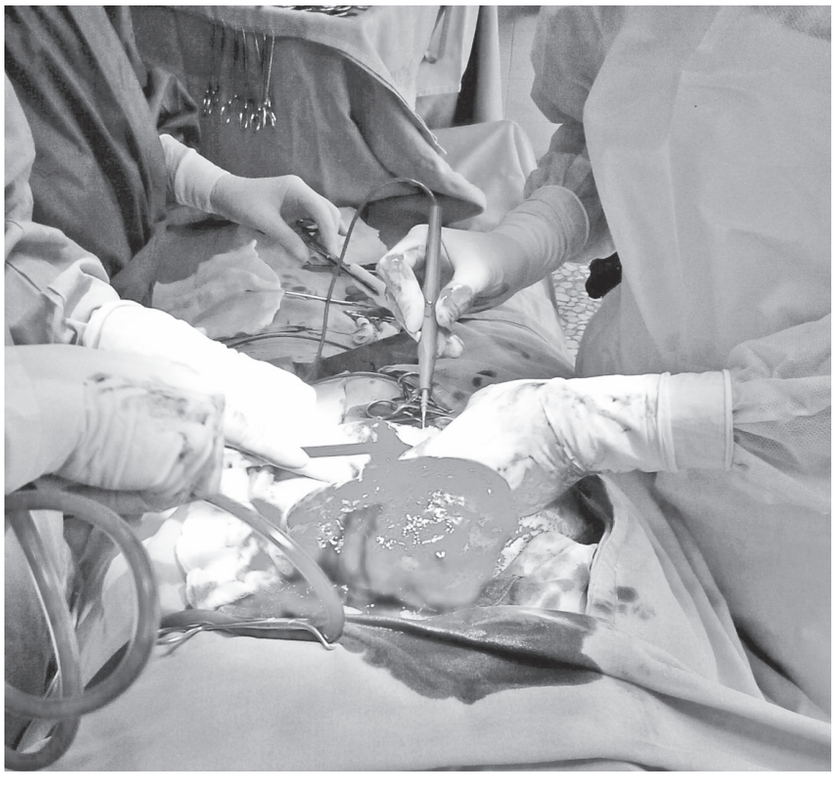

Figure. 1. ***The tumor still not extirpated and the pump aspirating the blood***

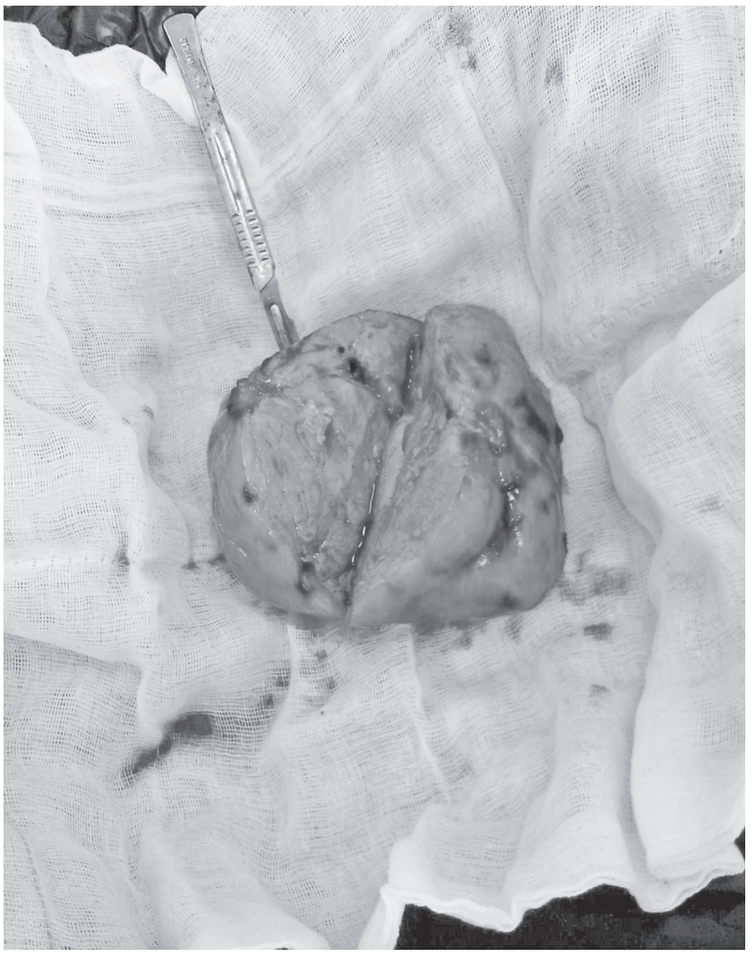

Figure 2. ***The tumor as a specimen with a scalpel next to it in order to understand how big it is***

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Мултидисциплинарен подход към пациенти със сепсис - това ли е най-добрият начин те да бъдат лекувани хирургично?

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Multidisciplinary approach to a septic patient - is that the best way to treat such cases?

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РЕЗЮМЕ:

Когато екип от лекари трябва да лекува пациент който има нужда едновременно от операция на сърцето и на заболяване в коремната кухина то той е поставен пред трудно решение – да се направи първо сърдечната операция, да се направи първо коремната операция, какъв да бъде интервала между двете в дни или да направи двете операции едновременно. Аз докладвам начина по който ние лекувахме такъв пациент с преимуществата и неудобствата на такова лечение. Правя преглед на начина по който други са решили същия проблем с плюсовете и минусите на тяхното решение.

Ключови думи: септичен пациент, мултидисциплинарен подход, кардио- и коремна операции

SUMMARY

When a team of doctors faces a patient that needs both an operation on his heart and his abdominal cavity they have to make a difficult decision – to operate the heart first, to do the abdominal operation first, how many days between the two operations is the best interval or performs both simultaneously. We report the way we treated such a patient, the advantages and disadvantages of such a treatment. We do a review of the manner of other teams have solved the same problem with the advantages and disadvantages of their decisions.

Key words: septic patient, multidisciplinary approach, cardio- and abdominal operations

INTRODUCTION

The entrance gate of infective endocarditis is not always clear. Shasha et al (9) report a rare possibility – cat scratch disease that led to infective endocarditis and hepato-splenic abscesses. Ozkurt et al (5) share another rare possibility – catheter-related nosocomial infective endocarditis due to methicillin-resistant Staphylococcus aureus complicated with splenic abscess in a pregnant woman. Patients on maintenance hemodialysis may develop bacterial endocarditis and splenic abscesses – such case is described by Kim et al (2). Peripheral and central septic embolization is a frequent complication in patients with active infective endocarditis affecting most commonly organs like brain, kidneys and spleen.

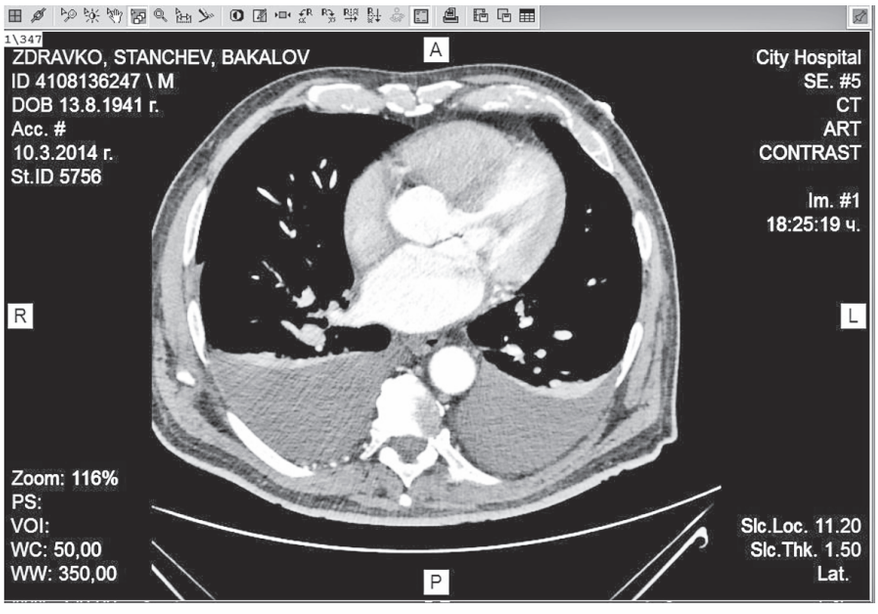
Splenic abscess as a complication of infective endocarditis is rare – 2-3% according to Mestress et al (4), 3-5% according to Mansur et al (3). A successful outcome lies with the choice between medical and surgical treatments, but if they remain untreated they lead to death. The discussion is still not closed about to treat them medically, to do splenectomy first, to do valve replacement first or perform both at one and the same time. The order of splenectomy and valve replacement might influence the outcome. Each has its own problems. Ryo Naito et al (8) quote an investigation published 20 years ago by Robinson et al about the data taken from 27 patients. They showed that medical treatment alone resulted in poor outcome while treatment with splenectomy resulted in high survival rate - 85%. According to the authors this is the largest clinical research published until 2010. Splenectomy is thought to be essential for eliminating the potential for prosthetic valve infection after valve replacement. But problems may be encountered with the development of an immuno-compromised condition and a tendency for bleeding. If valve surgery is done first prosthetic valve infection might occur because of existence of splenic abscess. Physical stress might be considerable in cases of double operation.

Ryo Naito et al (8) summarized 6 reports all the data are on 32 cases, 19 patients of them treated with therapy preceded by splenectomy, 10 with valve surgery first and 3 were treated simultaneously. The survival rates were 84.2% (16/19), 70% (7/10) and 100% (3/3). Doing splenectomy by open surgery or as a laparoscopic procedure is another theme for discussion. Laparoscopic procedure is less invasive, but the chance to open the abscess and spread it into the peritoneal cavity is higher.

Against laparoscopic procedure are the data reported by Gananadha S. and Leibman S. (1) for spontaneous rupture of the spleen as a complication of bacterial endocarditis. Laparoscopic surgery requires advanced techniques and institutions that are capable of performing the procedure are limited. Robert A. McCready et al (6) report a case of both infected splenic artery aneurysm and splenic abscess secondary to bacterial endocarditis. With advance in the understanding of immunologic role of the spleen there is a trend to preserve the spleen during treatment. A percutaneous drainage guided either by cross-sectional tomography (CT) or by ultrasound is attempted, but is not always successful. Robles et al (7) share a case they treated with successful percutaneous drainage guided by CT. The patient remained febrile and a new CT scan revealed residual splenic abscess. A splenectomy was performed in order to cure him.

CASE REPORT

ZSB, 72 years old male was admitted in City Clinic-Sofia on the 10 of March 2014. His illness started since November 2013 with a long lasting virus infection. He was febrile up to 38.5 C in the evenings; he had weakness, shivering and sweat. The patient was on antibacterial treatment – antibiotics were changed several times. His condition worsened – he had shortness of breath and fatigue. He fainted on the 1 of March 2014 and was admitted in a cardiology department. In that department was diagnosed infective endocarditis – vegetations on the mitral valve. Streptococcus parasanginis was identified by blood culture examination. Treatment with vancomycin, amikacin and tavanic was started and the patient was transferred to City Clinic for valve replacement. A systolic murmur 3/6 was present at cardiac apex. Laboratory studies disclosed leukocytosis – white cell count 19.65



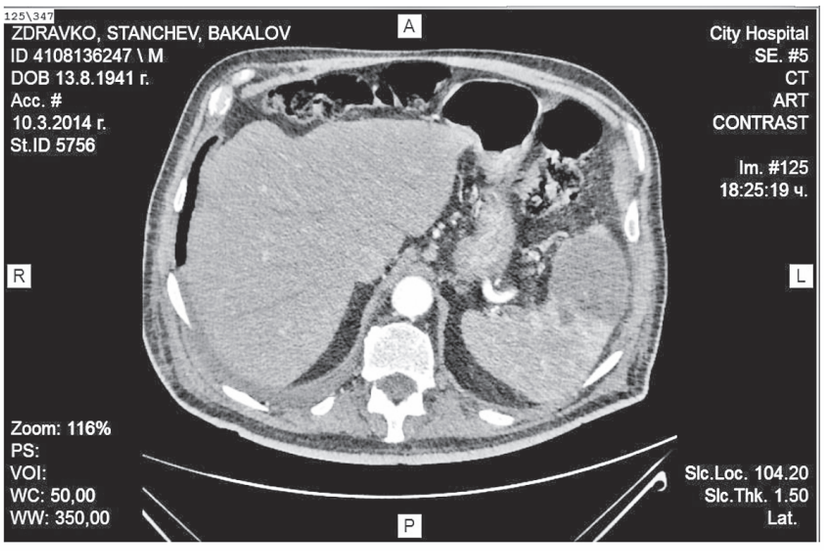
neutrophil 16.48; Hb 92.0; Er 3.72. Echocardiography identified vegetation on mitral valve 2.5/2 cm, mitral insufficiency 4-th degree. Right ventricular systolic pressure not directly measured was 65 mm Hg. Abdominal ultrasound suspected abscess in the spleen. It was enlarged 15.3/6 cm with a low attenuation area near the hilum 2.5 cm in diameter. A small accessory lien was found. Effusion in both pleura - 800 cc in the right, 650 cc in the left.

CT confirmed the pleura effusions and the accessory spleen. The difference was that several lesions were found in the spleen and they were connected between themselves.

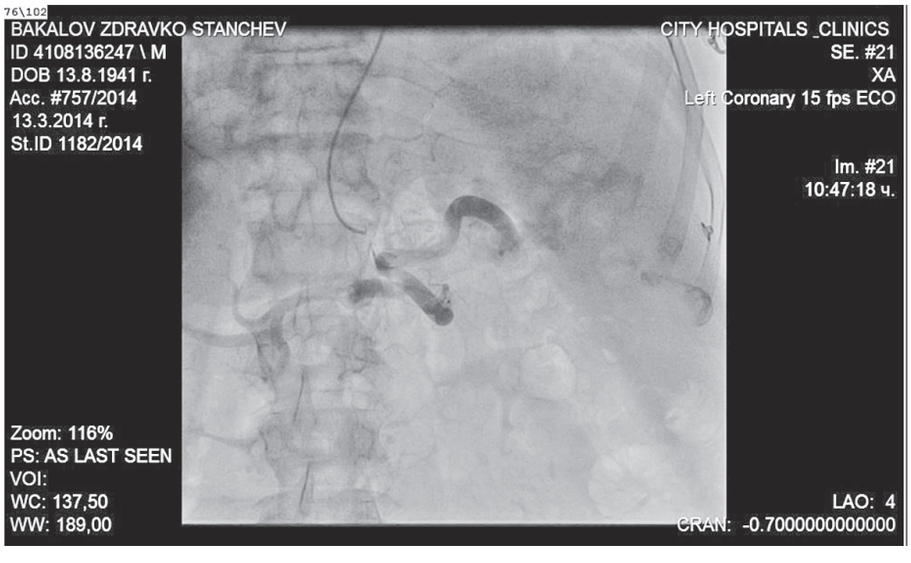
Never mind the high operative risk (EuroScore 32.46%) it was decided to operate. In one and the same day three things were performed: first embolization of the lien followed by open surgery splenectomy and at the end mitral valve replacement with a biological prosthesis Medtronic Hancock II Ultra № 31. Three different teams did the three different procedures. The clinical course after these was free of trouble.

DISCUSSION

The entrance gate in this case is clear. It is bacterial infection on the bases of a long lasting virus infection. For me, that means that such patients should be checked not only for bacterial pneumonia, but for infective endocarditis as well. In order not to have a delay in diagnose as has happened in the case I report – diagnose was made about 4 months after the first onset of symptoms. Of course, infective endocarditis has not started with the first onset of symptoms, but still 4 months is a long time. The embolization makes the prize of treatment higher, but I see at least two advantages of such a decision. An enlarged, inflamed spleen with an abscess in it contains more blood than an organ without pathology. Doing an embolization of the lien ahead of removing it reduces blood loss significantly. Another advantage is that it facilitates hemostasis in the abdominal cavity, something very important before heparinizing the patient for the valve replacement. This two reduce the probability of postoperative complications that may raise the prize more than the embolization dose. The possibility of development of an immuno-compromised condition is reduced



when an accessory lien is present. We had that in mind when making the decision to operate or not. In the literature I found embolization done in case of infected artery aneurysm, but not in a case like the one I describe. Doing valve replacement at the same time makes impossible for septic emboli from it to disseminate into other organs like brain, kidney, liver etc. The chances for cure of the patient will dramatically go down if that occurs. I do not ignore the own problems each of the procedures has, but the result we achieved makes me think that the decision was correct. Still I think that problems like that should be decided patient by patient until sufficient experience has been accumulated to formulate guide lines.



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Списание Българска медицина е издание на Българската академия на науките и изкуствата (БАНИ), Отделение наука, Научен център по медицина и здравеопазване. Излиза 4 книжки годишно. Списанието е достъпно електронно на сайта на БАНИ, раздел издания.

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Етически съображения по отношение на самите изследвания: всички трудове, които отразяват клинични проучвания следва да имат подписано информирано съгласие от пациентите и получено разрешение за изследването от съответната Етична комисия в съответствие с Декларацията от Хелзинския комитет. Експерименталните проучвания следва да имат също разрешение от съответната Етична комисия и от Българската Агенция по безопасност на храните и лекарствата.

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