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ADIPONECTIN CORRELATION WITH BIOCLINICAL BENEFITS OF USING NATURAL THERAPEUTIC FACTORS IN KNEE OSTEOARTHRITIS

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Abstract

Context and objective. The new insights in the pathogenesis of osteoarthritis (OA) reveal the implications of adipocytokines. This study aims to analyze the correlations between the serum value of adiponectin and the clinical rehabilitation effects in patients diagnosed with knee OA, admitted and treated in the complex balneal resort of Techirghiol lake.

Subjects and methods. The prospective randomized clinical study included 23 patients in the study group, diagnosed with knee OA according to ACR criteria, and a matching control group of 23 subjects. Serum level of adiponectin (using ELISA technique), uric acid, triglycerides, cholesterol, HDL-cholesterol and clinical response using a visual analog scale (VAS) were evaluated in all patients on their admission day and after 10 days of balneal treatment. Control group benefited from the same procedures except for cold mud therapy and mineral water baths.

Results. Plasma adiponectin levels (23.73±6.44 ng/dL) were statistically higher ($p<0.05$) in the study group compared to the control group (18.15±6.49 ng/dL). The mean VAS in both groups was decreased ($p<0.005$) compared to the initial moment.

Conclusions. Cold peloidotherapy combined with physical therapy and balneal factors induces serum adiponectin elevation and improves knee pain in OA. Therapeutic properties of Techirghiol mud still need further research.

Key words: adiponectin, osteoarthritis, mud, knee, VAS, Techirghiol.

INTRODUCTION

In the past years, in medical literature, emphasis has been placed on implication of adipocytokines in inflammatory processes of the osteoarticular system as well as in osteoporosis (1). Both white and brown adipose tissue is producing interleukin (IL)-1 β, tumor necrosis factor (TNF)- α, IL-6, IL-8, IL-17, basic fibroblast growth factor, vascular endothelial growth factor (VEGF), leptin, resistin, adiponectin and probably many others.

Infrapatellar fat pad (IPFP), fat tissue situated intracapsular and extrasynovial in the knee joints, rich in vessels, nerve fibers, immune cells, produces all the above mentioned adipocytokines which can play a role in the initiation and progression of knee osteoarthritis (2, 3).

OA is a multifactorial degenerative joint disease characterised by damaged articular cartilage, modified subchondral bone architecture, formation of bone exostosis and inflammation of the synovia.

Even if OA is the most common so-called "non inflammatory" rheumatic disease, according to new research, inflammation plays an important role in its etiology, along with obesity, age, previous injuries etc. (4, 5).

In the last years, balneological research was directed towards the study of specific inflammatory cytokines involvement in musculo-skeletal disorders. Correlations between peloid therapy and inflammatory pathways, at molecular level, are established and new insights in the world of chondrocytes and osteoblasts are brought to light (6).

In general, adiponectin is considered a benefic adipokine because of its systemic anti-inflammatory effect based on reduction of T cell activation, of the seric levels of IL 6 and TNF-α, of its antiatherosclerotic properties etc. (7).

Medical data is contradictory regarding the role of adiponectin in osteoarticular disorders. There is proof that adiponectin decreases both in the serum and in synovial fluid along with the aggravation of OA (8) and also with the radiologic deterioration of hand OA. (9). Contrary to all that, there are other opinions

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that adiponectin is involved in the pathogenesis of OA with a proinflammatory effect at chondrocytes level and also in the degeneration of interstitial cartilaginous matrix (10-12). In chondrocytes, *in vitro*, this adipokine induces pro-inflammatory mediators such as nitric oxide, IL-6 and matrix metalloproteinase with catabolic effects (13, 14). Furthermore, one study revealed a higher level of adiponectin in the IPFP of the patients with knee OA, especially in advanced stages, than in the subcutaneous tissue (15).

Mud, a balneotherapeutic agent used for centuries, preserves its mysteries regarding its implications in the pathophysiology of osteoarticular system. The complex biochemical composition of Techirghiol peloid, with its rich microbial flora, represents a valuable therapeutic option in musculo-skeletal disorders.

In an already published paper we obtained proof of the influence of warm mud applications over the serum levels of adiponectin in patients with knee OA (16).

This study aims to evaluate the serum level of adiponectin and the rehabilitation benefits using a visual analogous scale for pain (VAS) under the effect of natural therapeutic factors after 10 procedures of cold mud applications (CMA) in knee OA. We also aim to find out if there are any correlations between adiponectin and uric acid, considered a pro-inflammatory articular factor, and if balnear treatment affects adiponectin's cardiovascular protective role, regarding lipid profile.

PATIENTS, MATERIAL AND METHOD

In our prospective randomized clinical study, we included 2 groups: the study group consisting in 23 patients, between 30 and 70 years old, diagnosed with knee OA, according to ACR criteria (17) and a matching control group of 23 patients. The patients were hospitalized in the Balnear and Rehabilitation Sanatorium of Techirghiol between 01.06.2016-01.09.2016.

All subjects signed a special informed consent with the agreement to participate in this study and approval of the sanatorium ethical committee was obtained.

Patients from the study group (cold mud applications group-CMAG) performed 10 complex physical and balnear therapy procedures, in Techirghiol specific area, consisting in hydrotherapy (general cold mud baths and salt bath in the lake Techirghiol), heliotherapy, electrotherapy (current of low, medium, high frequency, laser therapy, ultrasonotherapy, phototherapy), massage therapy, kinesiology; while the patients in control group (CG) performed the same therapy except for specific hydrotherapy (mud baths or salt baths in Techirghiol lake).

Serum adiponectin, uric acid, cholesterol, HDL-cholesterol, triglycerides and clinical response after natural treatment with sapropelic mud were evaluated in all patients as follows. All patients were evaluated on their admission day and after 10 days of balnear treatment. Blood samples were collected at admission, before treatment and at discharge, both determinations respecting an overnight 12 h digestive rest. Serum adiponectin was determined at admission and after 10 days of treatment. Specific reagents were used for the determination of human adiponectin using ELISA technique produced by "DRG Instruments GmbH", Germany, with normal reference values of adiponectin according to BMI in Table 1 (however, the producer considered that every laboratory should establish their own values).

Table 1. Adiponectin reference values according to BMI

Gender	BMI (kg/m^2)	Mean (µg/mL)	SD (µg/mL)
Men	< 25	15.9	4.0
	25-30	13.8	4.0
	> 30	13.3	2.8
	Total	14.5	3.9
Women	< 25	18.6	5.4
	25-30	18.9	8.6
	> 30	16.4	3.8
	Total	18.2	6.1

In parallel, the groups were analyzed in terms of age, gender, average age of the disease, body mass index and severity of pain in the knee joints assessed by visual analogue scale (VAS).

Exclusion criteria were: patients with erythrocyte sedimentation rate (ESR) > 40 mm/h; patients with skin disorders (chronic or acute), including skin lesions; with serious conditions such as: cardiovascular, respiratory, digestive, renal, neurological; cancer; presence of connective tissue disorders, pregnancy. Previous treatments were restricted as follows: Nonsteroidal Antiinflammatory Drugs (NSAIDs) and steroids at least 4 weeks before study entry; surgery (arthroplasty, arthroscopy, abrasion, etc.) performed at least 3 months before the study; administration of corticosteroid intra/periarticular at least 6 weeks before starting the trial or hyaluronic acid with at least 6 months before the study; treatment with opioids for at least 4 weeks before study

entry; physiotherapy, acupuncture, transcutaneous electrical nerve stimulation (TENS), massage and physical therapy at least 4 weeks before study entry; administration of sedatives, hypnotics, anticonvulsant and muscle relaxant at least 2 weeks before study entry.

Statistical interpretation of the results was performed by processing data using IBM SPSS 18.0. The results were analysed with mathematical methods in order to obtain an average of the values, standard deviation and also the standard error of the media (descriptive statistics in order to characterise discrete and defined continuous variables); parametric statistical tests (t-Test to compare media of two independent samples and t-Test to compare media of paired samples).

Normal distribution was confirmed for all studied variables by Shapiro-Wilk test ($p > \alpha = 0.05$). By assessing Skewness and Kurtosis coefficients resulted that distributions are symmetrical and they do not present an abnormal vaulting.

Correlations were considered according to the values of the r correlation coefficient (adapted after D.E. Hinkle, W. Wiersma si G.S. Jurs, 1988, p.118).

The values of adiponectin, VAS, cholesterol, HDL-cholesterol, triglycerides, uric acid measured in the first day of treatment were registered as "Adiponectin 1", "VAS 1" etc. At the end of treatment, after 10 days of balneotherapy, the values were registered as "Adiponectin 10", "VAS 10" etc.

Statistical measurements were presented in Table 1.

RESULTS

Presentation of the study groups

The two groups contained 23 patients each and were considered statistically similar in terms of demographic features and of associated co-morbidities, such as metabolic syndrome and diabetes which might have had an impact over adiponectin values (18).

- sex (60.9% females in CG, 65.2% females in CMAG, p>0.05),

- age (medium value in CG=56.17±12.2 years *vs*. 55.17±8.28 years in CMAG, p=0.747>0.05),

- medium value of BMI (29±4.64 in CG *vs*. 27.69±4.33 in CMAG; p=0.328>0.05),

- medium value of VAS scale at admission (5.43±2.52 *vs*. 5.45±1.87, p=0.974>0.05),

- values of adiponectin 1 (17.90 ±8.04 ng/dL in CG *vs*. 20.24±9.90 ng/dL in CMAG, p=0.198>0.05). Adiponectin values were correlated with BMI in both groups (p <0.001, r = -0.690, negative correlation in CG and p = 0.002, r = -0.609, negative correlation on CMAG),

- values of glycemia in day 1 (CG: medium value 111.60 ±19.21 mg/dL *vs*. 118.52±34.20 mg/dL, p = 0.40>0.05,

- proportion of diabetic patients (considered both from anamnesis and after a cut-off value of glycemia of 126 mg/dL, twice registered) was 21.7% in CG *vs*. 26.1% in CMAG, ($\lambda 2$=0.00, p>0.05),

- length of disease (in CG: 8.7% of the patients had a duration of 1-6 months, 17.4% had a duration of 6-12 months, 43.5% had a duration of 1-5 years, 13% had a duration of 5-10 years and 17.4% of more than 10 years, in the CMAG the percentages are: 4.3%, 13%, 52.2%, 17.4% and 13 %. All proportions compared revealed a p>0.05),

- regarding the presence of metabolic syndrome the control group was statistically similar with the study group ($\lambda 2$ =0.392; p>0.05),

- both cholesterol and HDL –cholesterol and uric acid values did not differ significantly between groups.

We divided time of illness in the following time intervals: 1 – 6 months, 6 months – 1 year, 1 to 5 years, 5 to 10 years and more than 10 years. Occurrence of pain was considered the time of OA onset (the main feature of knee OA). We found that the patients suffering from pain for 1 up to 5 years were in a bigger number than in the other time intervals.

In CMAG, adiponectin 10 (23.73±6.44 ng/dL) is significantly higher than in day 1 (20.24±9.90 ng/mL) (t = -2.919; df = 22; Mdiff = -3.482; p = 0.008).

The difference between adiponectin 10 in CMAG and the one in CG was significant, also, in favor

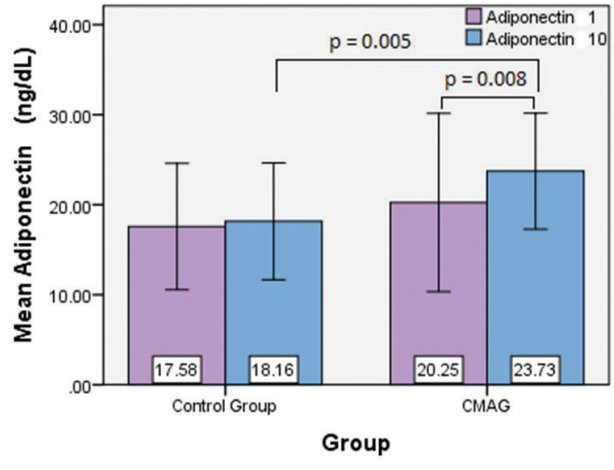

Figure 1. Values of Adiponectin 1 and 10 in both groups.

of the CMAG (t = -2.922; df = 44; Mdiff = -5.573; p = 0.005), see Figure 1.

We found no statistical difference between the values of adiponectin 1 and adiponectin 10 in CG (17.58±7.02 ng/mL *vs*. 18.15±6.49 ng/dL; t = -0.461; df = 22; Mdiff = -0.573; p = 0.649>0.05).

Adiponectin significant negative correlation with BMI persists in the 10th day of treatment.

VAS, a tool for measuring subjective pain that cannot be measured directly, was performed both in the research project baseline, day 1, and after 10 days of treatment.

VAS 10 in CG is 3.93±2.30 while VAS 10 in CMAG is 3.76±2.10.

Even if the difference between VAS 1 and VAS 10 is significant in each group (t = 4.657; df = 22; Mdiff = 1.5; p < 0.001 in CG, t = 6.28; df = 22; Mdiff = 1.69; p < 0.001 in CMAG), there is no difference between VAS 10 in CG and the one in CMAG (t = -0.267; df = 44; Mdiff = 0.17; p = 0.791).

In our study, VAS 10 had a significant negative correlation with adiponectin 10 value only in the CMAG (p = 0.036< α = 0.05, r = -0.44, considered a mild correlation).

DISCUSSION

The variety of the natural therapeutic agents used in balneotherapy, the influence of seasons and of the climate - different from the one of the patients' residence place, and of the changes in diet and daily activity, make strong scientific studies difficult in the field of balneology. Because of all that, debate exists in medical literature regarding the benefits of mud therapy.

In our study most of the patients were suffering from knee pain for 1 up to 5 years. It seems that patients with symptoms for less than 1 year are underestimating the knee OA evolution and prognosis, while those with more than 5 years of suffering are somehow "adapted and consoled" with the situation.

VAS is a psychometric response scale that can be used in questionnaires. It is a tool for quantifying subjective characteristics or attitudes that cannot be measured directly. When responding to a VAS item, respondents specify their level of agreement to a statement that indicates a position along a continuous line between 2 end points (pain with intensity from 1 up to 10, for example). In our study VAS was significantly lower on the 10th day of treatment in both groups. This is, once more, the proof of balneal treatment efficiency in knee osteoarthritis.

Our study brings proof not only of mud therapy efficiency but also of its possible correlations with adiponectin.

Adiponectin, an adipocytokine discovered in 1995, produced by adipocytes synthesis in many forms: low-molecular weight trimers, medium and high-molecular weight trimers, plays an important role in metabolic regulation and in inflammatory/anti-inflammatory processes. Among these, adiponectin enhances 5' AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor (PPARα) pathway in the striate muscle and in the liver, prevents insulin resistance by increasing fatty acids oxidation, offers cardio-vascular protection by augmenting the endothelial nitrous oxide production and reducing platelet aggregation, while its reduction seems to increase the risk of coronary heart disease, steatohepatitis, insulin resistance (19) as well as of biliary sludge and lithiasis (with similar contribution as other risk factors: pregnancy, parity, dyslipidemia etc) (20, 21).

If up to 5-6 years ago, medical data sustained the pro-inflammatory effect of adiponectin, with a deleterious effect over the cartilage (22-24) and just a few considering that adiponectin may have a protective role in the progression of OA, proving that both the serum and the synovial level of adiponectin decrease significantly with the severity of osteoarthritis (25), or that adiponectin modulates cartilage destruction in chondrocytes (up-regulating TIMP-2 and down-regulating IL-1beta- induced MMP-13) (26), new studies (27-29) are sustaining the beneficial effect of adiponectin over joint cartilage. Under the same light, the study of Zheng S *et al.* published in 2016 in Scandinavian Journal of Rheumatology sustaines that serum adiponectin is significantly and negatively associated with radiologic severity of knee osteoarthritis (30).

Looking at our results, we found a statistically significant variation of adiponectin after 10 days of complex balneal therapy in the study group compared to control group. Cold mud bath induced a significant increase in serum adiponectin values both related to value of adiponectin 1 in CMAG and to the adiponectin 10 of the control group. Based on our previous study, in which we found that hot peloid bath induced significant decrease in serum levels of adiponectin value after 10 days (16), we suppose that thermoregulation interferes with therapeutical results of peloid baths, along with other similar results from medical literature (31, 32). This is why we will extend our study with repeated

dosage of serum levels of adiponectin at 3 months/ 6 months after balneal contrastant treatment, because we have a new work hypothesis.

By excluding the possibility of a pregnancy, in fertile patients, we, first, reduced the necessary investigations, some of them being rather specific for our region (33). We have, also, took care of the possible adverse effect of anticonvulsant therapy – even before the 4 weeks prior the beginning of the study (34). A possible effect of the cortisol, due to high estrogen levels (35) was also avoided.

We did not find a significant correlation between adiponectin values and the levels of uric acid, HDL-cholesterol, triglycerides in either group.

The results of this study, that adiponectin values are higher in case of cold mud applications than in the control group, are in contrast with our previous results (16), in which hot mud baths reduced the value of adiponectin. At the same time the significant negative correlation between VAS scale and adiponectin level is pleading for a protective role of adiponectin over knee osteoarthritis. So, we consider that thermoregulation is involved, and further research is needed to establish the relation between adiponectin, reduction of pain and cold peloid treatment with Techirghiol mud.

In conclusion, although the study included a small number of cases, it allowed the issuance of plausible assumptions on understanding the beneficial effects of Techirghiol peloid on osteo-articular degenerative disorders.

In our study we find a significant elevation of adiponectin level after 10 cold sapropelic mud applications in the study group as related to the control group.

Even if prevention of obesity related to pro-inflammatory cytokines' production is still relying on physical activity, reduction of fat body mass and healthy diet, new data about control of adipokines and their effect over osteoarthritis may represent the base of new pharmacological approaches in this disease.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contribution

Ionescu Elena-Valentina and Tica Irina equally contributed to this article.

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HYPOCALCEMIC CARDIOMYOPATHY - A RARE HEART FAILURE ETIOLOGY IN ADULT

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Abstract

Introduction. Heart failure and dilated cardiomyopathy (DCM) in adults are rarely caused by hypoparathyroidism induced hypocalcemia.

Case report. Female patient, 40 years old, diabetic, with previous history of thyroidectomy for Graves' disease, was hospitalized for syncope and symptoms of heart failure. ECG revealed sinus tachycardia, long QT, negative T from V1 up to V4. Chest X-ray, cardiac ultrasound and contrast cardiac MRI confirmed dilated left chambers, severe systolic dysfunction of the left ventricle (left ventricle ejection fraction=15%) due to diffuse hypokinesia and restrictive type of diastolic dysfunction. Patient status insignificantly improved with specific heart failure depletion treatment but important signs of hypocalcemia occurred. Low levels of total and ionic serum calcium were detected (total serum calcium 3.6 mg/dL, ionic calcium=2.2 mg/dL) along with low serum levels of parathormone (10 pg/mL) and high level of phosphatemia (6.4 mg/dL). After one month of parenteral treatment with calcium and oral vitamin D, hypocalcemic signs disappeared and heart failure significantly improved.

Conclusion. This rare adult condition is refractory to heart failure conventional therapy but promptly responds to restoration of normocalcemia. It is important to be aware of this pathophysiological setting, in order to treat it correctly.

Key words: hypocalcemia, hypoparathyroidism, heart failure, dilated cardiomyopathy.

INTRODUCTION

Calcium is an essential element for the normal ventricular systolic and diastolic function. Over the active process of myocardial membrane depolarization, there is a rapid inflow of calcium ions via active (voltage-gated) membrane calcium channels, and subsequent calcium ions' release from sarcoplasmic reticulum (1). Then calcium binds with the troponin-tropomyosin complex, supporting the myocardial contraction: actin-myosin sliding and jointing. Relaxation occurs when calcium ions are actively pumped back into sarcoplasmic reticulum, tropomyosin resumes its shape and myosin slides back and uncouples actin (2).

Therefore, it seems obvious that hypocalcemia will affect cardiac contractility, and this was proved in current studies (3). Meanwhile, recent evidence suggests that vitamin D and parathormone (PTH) may have an independent role to play, also (4, 5).

Hypocalcemic heart failure is a rare finding in clinical practice. It was described especially in infants, due to severe vitamin D deficiency (6-8). Hypocalcemic ventricular dysfunction and dilatation in adulthood is substantially less found and described, being mainly related to hypoparathyroidism (9-11). This condition is refractory to heart failure conventional therapy, but if the etiology is recognized, it responds promptly to restoration of normocalcemia.

CASE REPORT

We report the case of a 40-year-old woman without cardiovascular history, who presents in Emergency Care Unit for syncope, progressive exertional dyspnea up to dyspnea at rest and paroxysmal nocturnal dyspnea. She is known with type 1 diabetes mellitus and with thyroidectomy for Graves' disease for 6 years, with levothyroxin substitution treatment. Patient's written approval was obtained in order to publish her case.

At admission the patient was conscious, in moderate distress, with normal body temperature, spontaneous peripheral oxygen saturation of 93%, blood pressure 100/60 mmHg equal in both arms, a regular ventricular rate of 100 beats per min, with a holosystolic IIIrd/IVth degree murmur in the axilla, protodiastolic left ventricle gallop, mild peripheral edema, bilateral lung stasis rales, moderate hepatomegaly (inferior liver limit at 3 cm below the costal edge).

Her ECG revealed sinus tachycardia at 100/

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min, QRS axis at +90 degrees, long QT (QTc=640 ms), negative T from V1 up to V4 (Fig. 1).

Laboratory analysis at admission revealed: elevated glycaemia (270 mg/dL), elevated liver enzymes, elevated N-terminal pro b-type natriuretic peptide (NT-proBNP), hypercholesterolemia, markers of myocardial necrosis within physiologic limits, with no rising pattern, elevated TSH (Table 1).

The chest X-ray exhibited signs of pulmonary stasis with hilum enlargement due to vascular elements involvement and an increased cardio-thoracic index.

Cardiac ultrasound disclosed dilated left chambers (left ventricle end-diastolic diameter: 57 mm, left ventricle end-systolic diameter: 51 mm), left ventricle (LV) severe global systolic dysfunction (LVEF=15%) due to diffuse hypokinesia, restrictive type of diastolic dysfunction with elevated filling pressure indexes (E/E'=19), moderate functional mitral regurgitation, IIIrd degree tricuspid regurgitation, moderate pulmonary hypertension (peak systolic trans-tricuspid pressure gradient of 40 mmHg, systolic pulmonary artery pressure of 50mmHg), pericardial circumferential effusion of maximum 3 mm (Fig. 2). Angiocoronarography was performed, in order to exclude a plausible ischemic LV failure. This investigation revealed permeable epicardic coronary arteries, with no atherosclerotic lesions (Fig. 2).

Cardiac MRI was also performed, proving homogeneous myocardial perfusion and a late contrast enhanced aspect within normal limits (Fig. 3).

Treatment was initiated with loop diuretic (furosemide 120 mg/24 h i.v., decreasing progressively to a daily dose of 40 mg p.o.), ACE inhibitor (ramipril 2.5 mg/ day), beta-blocker (carvedilol up to 6.25 mg/ day), anti-aldosterone diuretic (spironolactone 25 mg/ day), atorvastatin 20 mg/day, insulin and levothyroxine (titered up to 150 microg/day).

Giving the circumstances of LV dilatation with severe systolic dysfunction and the presence of long QT – we presumed an episode of tachyarrhythmia as a cause for syncope. Repeated 24 hours ECG monitoring showed no significant arrhythmias.

Patient's cardiac status was very slowly improving under treatment, with partial remission of the congestive signs, 6 kg loss in weight, residual dyspnea at moderate effort, but she started to complain of important nocturnal muscular cramps and parestesia. Along hospitalization, she started to exhibit typical hypocalcemic signs of neuromuscular hyperexcitability: Chvostek, Weiss, Trousseau positive

Table 1. Biochemistry and hormonal values

Day	Admission	Discharge
Glycemia (nv 60-110 mg/dL)	270	109
ALAT (nv<31 U/L)	361	105
ASAT (nv<32 U/L)	463	83
GGT (nv<40 U/L)	909	503
Uric acid (nv: 2-5.7 mg/dL)	4.5	
Serum cholesterol (nv: <200 mg/dL)	322	213
HDL-Cholesterol (nv>40 mg/dL)	97	34
LDL-Cholesterol (nv<100 mg/dL)	223	163
Triglycerides (nv:<150 mg/dL)	187	116
Creatinine (nv: <1 mg/dL	0.91	1
NT-proBNP (nv<86 pg/mL)	900	
CK-MB (nv<24)	17	22
Troponin T (nv<14 pg/mL)	12.42	12.21
Na (nv: 134-145 mEq/L)	134	143
K (nv: 3.3-5.1 mEq/L)	5.3	4.5
Anti dsDNA (nv: <100 UI/mL)	54	
Anti Ro antibodies: (nv: <7 U/mL)	4.2	
ANCA: (nv: <1/10)	1/25	
Total serum calcium (nv: 8.6-10 mg/dL)	3.6	5.2
Ionic serum calcium (nv: 3.82-4.82 mg/dL)	2.2	2.9
Serum magnesium (nv: 1.6-2.6 mg/dL)	1.55	1.8
Serum phosphorus (nv: 2.5-4.5 mg/dL)	6.4	4.3
Parathormone (nv: 15-65 pg/mL)	10	6.7
TSH (nv: 0.27-4.20 μUI/mL)	15	5.6
25-OH-vitamin D (nv: 30-100 ng/mL)	21.8	32

ALAT= Alanine aminotransferase; ASAT= Aspartate aminotransferase; CK-MB= Creatine kinase-MB; NT-proBNP = N-terminal pro b-type natriuretic peptide dsDNA = anti-double stranded DNA antibodies; ANCA = anti-neutrophilic cytoplasmic antibodies. TSH= Thyroid stimulating hormone.

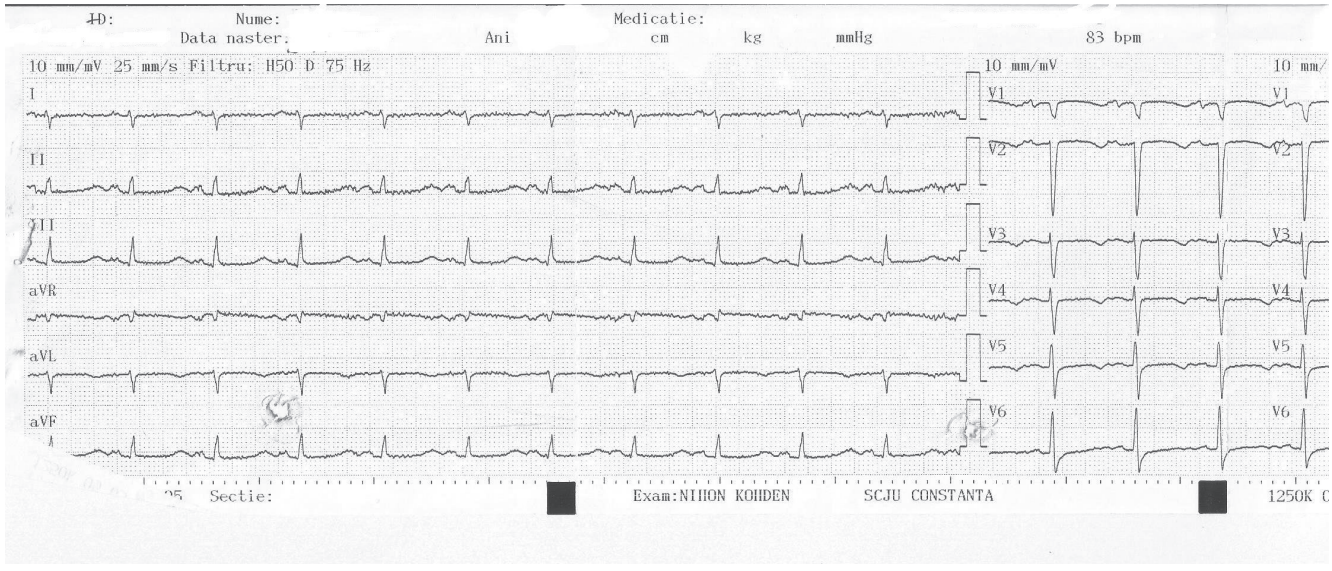

Figure 1. ECG at admission.

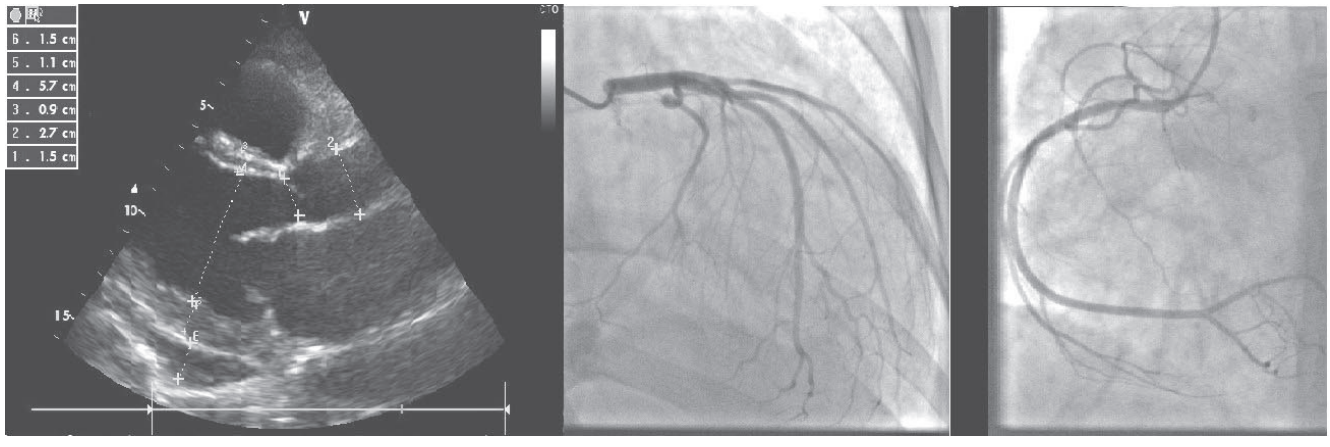

Figure 2. Cardiac ultrasound and angiocoronarography (left and right coronary arteries without atherosclerosis).

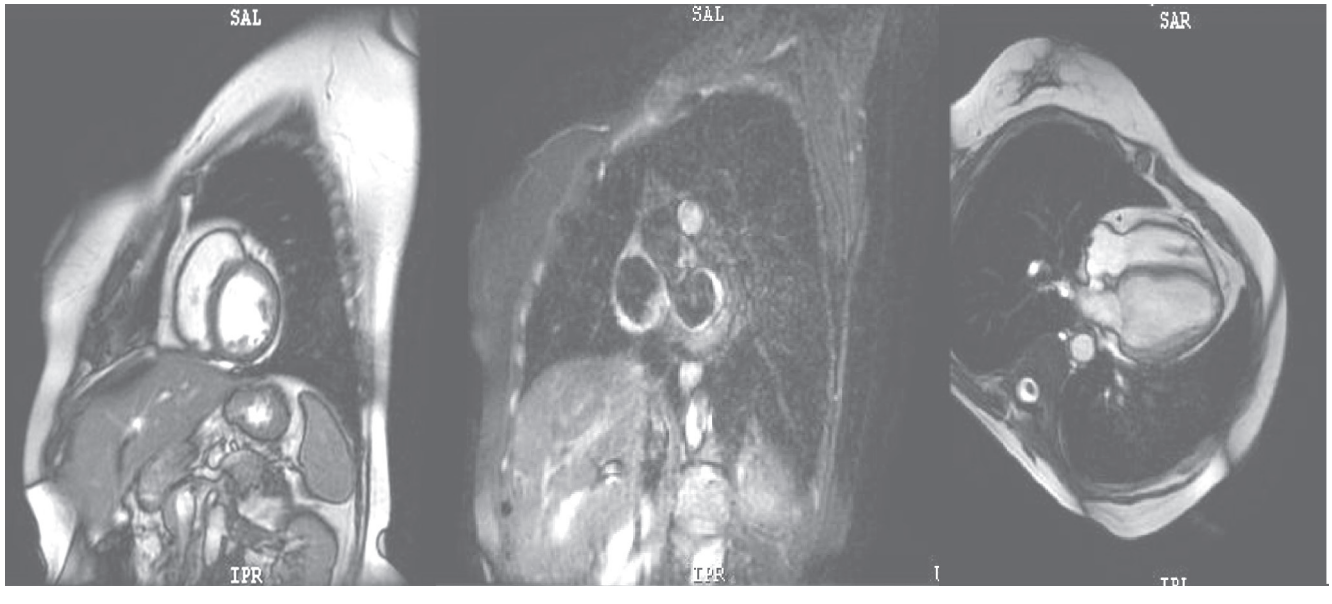

Figure 3. Cardiac MRI: homogeneous myocardial perfusion within normal limits.

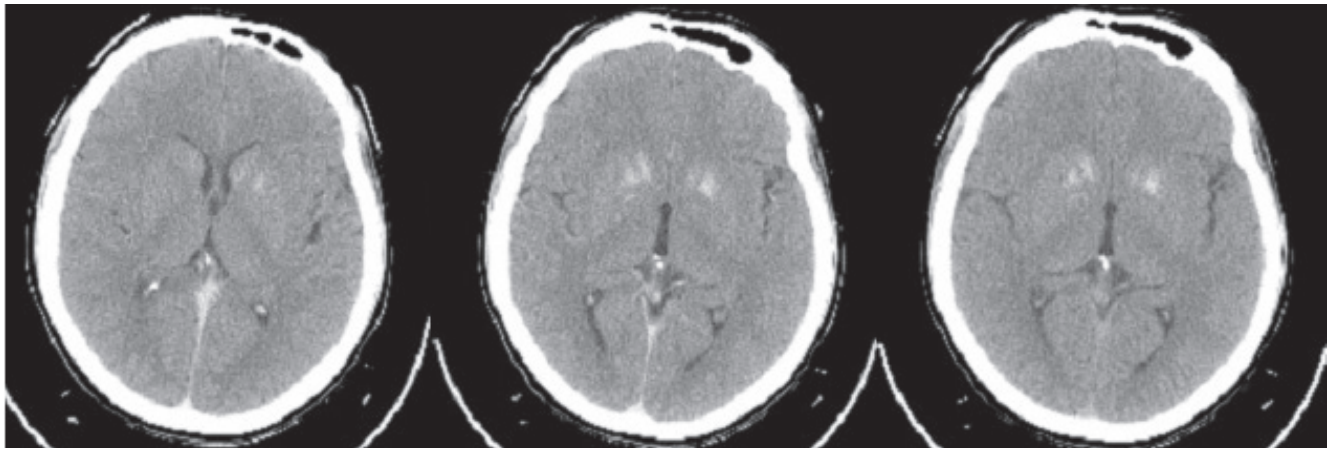

Figure 4. Cerebral CT scan revealing calcification of the basal ganglia.

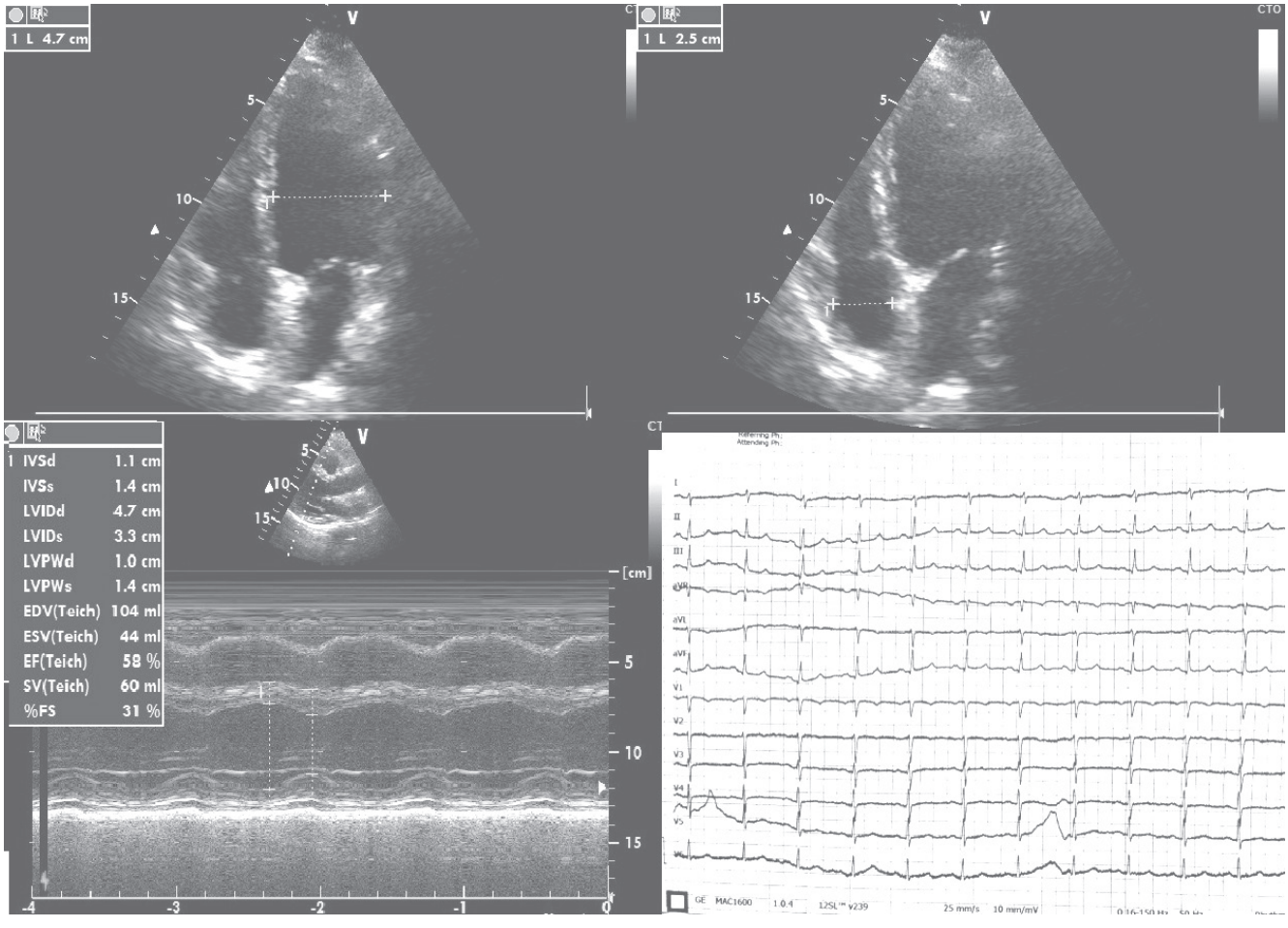

Figure 5. Normal cardiac ultrasound and ECG one year after discharge.

signs, psychomotor agitation and convulsions.

Laboratory blood tests disclosed severe hypocalcemia: total serum calcium of 3.6 mg/dL, ionic calcium=2.2 mg/dL. So, we considered hypocalcaemia pre-existent to the actual cardiac distress, based not only on the anamnesis but also on the long QT segment at admission, that it was curiously symptomless before, and that it was exacerbated by the diuretic treatment.

Parathormone testing was assessed, along with phosphoremia, and they revealed severe primary hypoparathyroidism (Table 1).

Further investigation was performed: cerebral CT scan that revealed calcifications of the basal ganglia. (Fig. 4).

Parenteral treatment with gluconic calcium was started: 950 mg of calcium (1 ampoule) at 6 hours, followed by oral treatment – lactic calcium 6000 mg/day and calcitriol 0.5 μg per day was associated. The following days patient's clinical and serological evolution improved, hypocalcemic signs have disappeared in several days.

Patient was discharged after one month with important improvement of cardiac symptoms (heart failure regression from IVth NYHA class to IInd NYHA class), improvement of LVEF (30%) with no hypocalcemic manifestations and with the recommendations to continue cardiologic treatment and lactic calcium supplementation up to 6 g per day along with 2 μg of alpha-calcidolum. She was advised to monitor her serum calcium levels every two weeks in the first two months, along with regular kidney ultrasound, and every three months afterwards.

One year after the above-mentioned discharge, the patient was under daily medication with 3 g lactic calcium, 2 μg of alpha-calcidolum, 12.5 mg carvedilol and 150 μg levo-thyroxine, and complained only of dyspnea at intense efforts with no hypocalcemic symptoms. She had normal values of calcemia and 25-OH-vitamin D. Control cardiac ultrasound and ECG were performed and they both were within normal limits (Fig. 5).

DISCUSSION

Identifying the etiology of DCM in order to establish the optimal therapy is of great importance, due to the fact that certain types are reversible (ethanolic DCM is reversible when alcohol consumption stops, tachyarrhythmia induced DCM is reversible when tachycardia disappears). In the meantime, genetic primary dilative cardiomyopathy has no etiological treatment, only some promising new therapies: stem cells transplant, genic therapy and micro-RNA (12).

Even if epicardial coronaries are permeable we still could not exclude the coronary heart disease: hyperglycemia may induce ischemic disorder due to lesions of the microvascularisation. These lesions cannot be revealed either by coronarography or by angio CT scan (13).

Dysglycemia and insulin resistance may directly damage the atrial and ventricular myocardium by inflammatory mechanisms (14). Medical literature sustain that it is reasonable for the cardiovascular phenotype to get worse as glucose impairment is more severe, hypothesizing that geometry and function of the heart are gradually affected by progressive deterioration in glucose metabolism (15).

Our patient has no familial history of DCM and no recent occurrence of fever. Nevertheless, clinical manifestations as well as the young age of our patient can induce the suspicion of a previous myocarditis evolving towards DCM. Anyway, cardiac MRI proved homogeneous myocardial perfusion and a late contrast enhanced aspect within normal limits.

Another hypothesis in our case was a form of cardiac damage due to an autoimmune disease (16). Arguments were: the feminine gender, the young age and the presence of already two autoimmune disorders: type 1 diabetes mellitus and Graves' disease. To rule out this possibility we performed dosage of anti-double stranded DNA antibodies (anti dsDNA antibodies), anti Ro antibodies and ANCA (anti-neutrophilic cytoplasmic antibodies) – all of them negative (see Table 1). More than this, there was no clinical evidence for a systemic disorder.

Tachyarrhythmic cardiopathy was not taken into account – because the hyperthyroidism of Graves'disease, that usually induces sustained tachycardia, was solved 6 years ago and the patient's thyroid status at admission was hypothyroidism. The tachyarrhythmic dilation of LV usually improves several months after controlling the ventricular rate (17).

Neuromuscular symptoms completely remitted after calcemia normalized. Investigation of hypocalcemia in our patient revealed the diagnosis of primary hypoparathyroidism, confirmed both by serum values of total and ionic calcium, serum parathormone and by basal ganglia calcification in CT.

The acquired hypoparathyroidism was due to a lesion of the parathyroid glands' vasculature during thyroidectomy and not to accidental parathyroidectomy, according to the histopathological examination.

Hypoparathyroidism is an uncommon condition due to a large spectrum of etiologies: congenital or acquired. Congenital causes include Di George syndrome (parathyroid hypoplasia, cardiac, renal and skeletal disorders associated with neurocognitive impairment and thymus hypoplasia), autoimmune polyendocrine syndrome type 1 (adrenal insufficiency, candidiasis and hypoparathyroidism), inherited genetic mutations of the pre-proPTH gene. Acquired causes include surgery (during thyroidectomy, neck dissection, parathyroidectomy), autoimmune hypoparathyroidism alone or part of an autoimmune polyglandular syndrome or isolated non-genetic hypoparathyroidism (very rare) (18).

PTH plays an important role in cardiac physiology not only due to calcium homeostasis but also directly, as recent molecular studies revealed (19). In adult cardiomyocytes, PTH directly induces a calcium influx, by activating G-protein signaling. Calcium influx will activate protein kinase C which, interfering with adrenoreceptor B, will attenuate contractility, and not induce a direct contractile effect, as presumed. PTH exerts changes in cardiac myocyte proliferation and hypertrophy too (5). In the meantime, PTH decreases the calcium influx in vascular smooth muscle cells, with subsequent vasodilation (20).

Although the exact mechanisms are not known, hypocalcemia seems to reduce cardiac contractility, while PTH itself seems to have a positive chronotropic effect on cardiomyocytes (21).

A peculiarity of our case was the possible long evolution of subclinical hypoparathyroidism as the patient suffered thyroidectomy 6 years ago and she was never suspected with hypoparathyroidism. We found only one similar case, with such a late revealed hypoparathyroidism after thyroid surgery, in medical literature (10).

In conclusion, especially because of the rarity of hypocalcemia induced dilated cardiomyopathy in adults, this etiology must be suspected in a cardiac patient with neuro-muscular signs as parestesia, neuromuscular hyperexcitability or convulsions.

Early calcium supplementation, usually along with vitamin D, reduces and even reverses ventricular hypertrophy and dilatation as well as conduction abnormalities (5), as it was our case.

Conflict of interest

All authors equally contributed to this paper and they declare no conflict of interest.

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Brain - Derived Neurotrophic Factor - a Marker for the Balneal Treatment of Chronic Low Back Pain?

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Up till now, to our knowledge, there are no studies evaluating serum Brain - Derived Neurotrophic Factor (BDNF) levels in patients with degenerative chronic low back pain under rehabilitation treatment. BDNF is a neuroprotein associated with neuropathic pain and represents an important mediator of the effects of physical exercise. Complex balneal treatment with hot Techirghiol sapropelic mud reduces pain and increases serum levels of BDNF in these patients.

Key words: degenerative pain, BDNF, balneal, sapropelic mud

The main cause of low back pain (LBP) worldwide is lumbar disc herniation (LDH). LDH, a degenerative disease which induces narrowing of the spinal canal, seems to be associated with several single nucleotide polymorphisms (SNPs). For example, seven SNPs were genotyped in a Chinese study performed over 1072 patients [1] and a relation was found between these SNPs and brain derived neurotrophic factors (BDNFs) and their genes (BDNFOs).

BDNF is part of the neurotrophin family of growth factors. They are related to the canonical nerve growth factor, playing an important role in the maturation and differentiation of neurons. Low levels of BDNF are associated with neurodegenerative disorders as Parkinson's disease, multiple sclerosis or Alzheimer disease [2]. Data in medical literature related to BDNF level and its involvement in LBP is controversial, some studies revealing elevated levels of BDNF after pain-relieving methods of physical therapy [3], some others: high levels of plasma BDNF in old women with LBP compared to pain-free controls [4].

Considering the growing burden of LBP worldwide, the studies over how balneal treatment affects neurotrophic factors involved in neural regeneration and protection after spinal cord injury or peripheral nerve disorders can represent the beginning of a new approach in rehabilitation and non-pharmacological therapies [5, 6]. Under these circumstances, we consider our study over the serum level of BDNF in patients treated for LBP with sapropelic mud from Techirghiol lake to be important and proving that balneal treatment significantly modifies the serum level of BDNF.

Experimental part

We present a prospective case-control cohort study, which included 50 patients hospitalized for 2 weeks in the Balneal and Rehabilitation Sanatorium of Techirghiol (BRST). Patients signed an informed consent and the study was approved by the ethical committee of the Sanatorium. Patients with degenerative chronic LBP, with indications for balneal treatment, both women and men, were included in the study. Exclusion criteria were: any inflammatory diseases, high blood pressure, cardiac failure, any pulmonary, renal, endocrine, neurologic or oncologic diseases, skin lesions, any antidepressant treatments. Patients were divided into two groups: hot mud bath group (HMBG) and a statistically matched control group (CG). Patients in the first group benefited from hot mud baths treatment and complex rehabilitation treatment such as electrotherapy, kinetotherapy and massage therapy. The control group had the same treatment, except for the hot mud baths. Each patient was clinically tested before and after treatment. Schober index, finger ground distance (IDS) index (for evaluation of lumbar spine mobility) and the visual analogue scale (VAS) for evaluation of pain were monitored. For each patient blood samples were collected in the beginning and in the end of treatment in order to determine the BDNF serum levels For quantitative detection of BDNF we used a sandwich high sensitivity Eliza kit: Human BDNF PicoKine form Booster Biologic Technology (USA) and Eliza reader StatFax 4700 Microstrip Reader Awareness Technology (USA) Blood prelevation was performed in the same conditions for every patient and laboratory testing was performed by the same doctor. Patients completed a demographic questionnaire regarding age, gender, residence, personal history of pain, body mass index (BMI), frequency of treatment in BRST (twice a year, as indicated, once a year, sporadically and for the first time). Hot mud bath therapy involved diluting 10-15 kg of sapropelic mud, from Techirghiol Lake, in a water tub. The patient was immersed in the hot mud bath for 20 minutes, at 38-39^{0}C under supervision of a physiotherapist.

Statistical evaluation of obtained data was performed using Wilcoxon test (level of significance: 0.05) for dependent samples, the nonparametric Mann-Whithney U test (level of significance: 0.05) for independent samples. Chi-Square test (with $p < \alpha = 0.05$ level of significance) and Spearman's rho were also used. Minimum, maximal, median and Interquartile Range values were obtained and compared.

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Results and discussions

Most of our patients were women, the median age, in both groups, was 54 years old and the majority of the patients included in the study were overweight and had urban residence.

The demographic features of our groups are detailed in Table 1.

We evaluated VAS scores at admission and discharge for each patient and we discovered significant lower VAS values at discharge than VAS values at admission. (Figure 1).

The Wilcoxon test confirmed statistically significant differences of VAS median values before and after treatment for CG ($p<0.001$) and for HMBG ($p<0.0001$), (Table 2).

The result of Wilcoxon test for Schober test values in HMBG patients confirmed statistically significant differences between the median values at admission and those at discharge $p<0.001$ (Table 3). Analyzing the *finger –ground index* for both groups at admission and discharge, we did not find any statistically significant differences (Table 3).

We evaluated BDNF serum levels for each patient at admission and at discharge (Table 4).

According to the non-parametric Mann-Whithney U test there were no significant differences between serum BDNF values of the two groups at admission ($p=0.123 > 0.05$) while at discharge the values significantly differed between groups ($p<0.001$) (Figure 3). In the same time the Wilcoxon test confirmed that within each group the values of serum BDNF at discharge were significantly different from the ones at admission ($p< 0.001$) (Figure 3).

According to Box-Plot representation (Figure 3) the BDNF values in control group were higher at admission than at discharge, while in HMBG the BDNF values were higher at discharge than at admission. We did not find any correlation between BDNF serum values and regular (once or twice per year) or sporadic balneal treatment. We did not discover any significant correlation of serum BDNF

		Hot Mud Bath Group	Control Group
Number		25	25
Age (years)		54.00±9.147 (39-68)	54.00±6.813 (41-67)
Gender	Male	7	10
	Female	18	15
BMI (Kg/m²)	18.50-24.99	9	15
	25-29.99	8	5
	30-34.99	5	2
	35-39.99	2	3
	≥40	1	0
Urban Residence		88% (22)	92% (23)
Frequency of balneal treatment:	biannual	12%	8%
	annual	48%	24%
	occasional	20%	32%
	First time	20%	36%
VAS score at admission		7.08±1.63	5.28±1.99
Quantified Schober index at admission		4.76±0.72	4.68±0.90

BMI=body mass index, VAS=visual analogue scale

Table 1
DEMOGRAPHIC FEATURES OF BOTH GROUPS

		Lot	
		CG	HMBG
VAS at admission	Minimum	2.00	5.00
	Maximum	9.00	10.00
	Median	5.00	7.00
	Percentile 25	4.00	6.00
	Percentile 75	7.00	8.00
	Mean	5.28	7.08
	Standard Deviation	1.99	1.63
VAS at discharge	Minimum	2.00	2.00
	Maximum	8.00	8.00
	Median	3.00	4.00
	Percentile 25	3.00	3.00
	Percentile 75	4.00	5.00
	Mean	3.96	4.32
	Standard Deviation	2.11	1.68

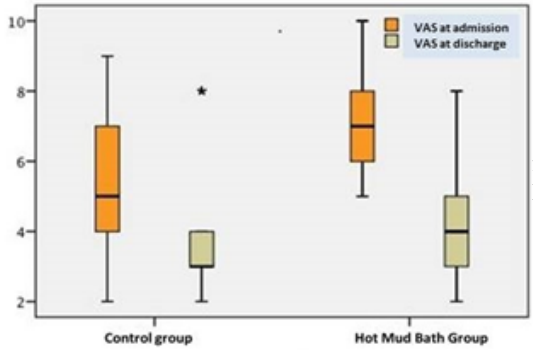


Fig. 1. VAS scores distribution for patients from the study groups and Box-Plot representation of VAS scores at admission/ discharge for each of the studied groups.

Group				VAS at admission	VAS at discharge
Spearman's rho	Control group	VAS at admission	Correlation Coefficient	1.000	.571**
			Sig. (2-tailed)	.	.003
			N	25	25
		VAS at discharge	Correlation Coefficient	.571**	1.000
			Sig. (2-tailed)	.003	.
			N	25	25
	HMBG	VAS at admission	Correlation Coefficient	1.000	.823**
			Sig. (2-tailed)	.	.000
			N	25	25
		VAS at discharge	Correlation Coefficient	.823**	1.000
			Sig. (2-tailed)	.000	.
			N	25	25

**. Correlation is significant at the 0.01 level (2-tailed).

Table 2
SIGNIFICANCE OF VAS VARIATIONS

Table 3

SPEARMAN'S RHO. SIGNIFICANCE OF SCHOBER'S TEST AND *FINGER-GROUND INDEX* VARIATIONS

			Schober at admission	Schober at discharge			"Finger – ground index" at admission	"Finger – ground index" at discharge
Control Group	Schober at admission	Correlation Coefficient	1.000	.787**	"Finger – ground index" at admission	Correlation Coefficient	1.000	.999
		Sig. (2-tailed)	.	.000		Sig. (2-tailed)	.	.000
		N	25	25		N	25	25
	Schober at discharge	Correlation Coefficient	.787**	1.000	"Finger – ground index" at discharge	Correlation Coefficient	.999**	1.000
		Sig. (2-tailed)	.000	.		Sig. (2-tailed)	.000	.
		N	25	25		N	25	25
Hot Mud Bath Group	Schober at admission	Correlation Coefficient	1.000	.526**	"Finger – ground index" at admission	Correlation Coefficient	1.000	.958**
		Sig. (2-tailed)	.	.007		Sig. (2-tailed)	.	.000
		N	25	25		N	25	25
	Schober at discharge	Correlation Coefficient	.526**	1.000	"Finger – ground index" at discharge	Correlation Coefficient	.958**	1.000
		Sig. (2-tailed)	.007	.		Sig. (2-tailed)	.000	.
		N	25	25		N	25	25

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4

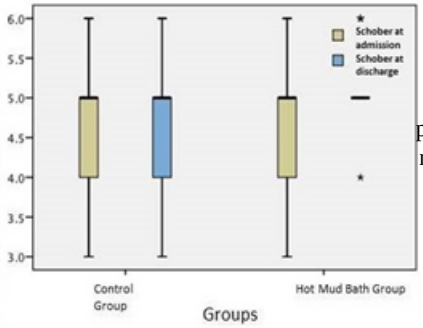
LEVELS OF SERUM BDNF IN CONTROL GROUP AND HOT MUD BATH GROUP

	Group			
	Control Group		HMBG	
	BNDF at admission (pg/mL)	BDNF at discharge (pg	BNDF at admission (pg/mL)	BDNF at discharge (pg/mL)
Minimum	318.90	39.50	346.00	1016.80
Maximum	1842.00	1698.00	1649.20	1998.00
Median	1416.00	498.70	1211.40	1646.70
Percentile 25	690.00	272.20	1051.00	1402.40
Percentile 75	1771.00	986.40	1304.00	1846.50
Mean	1257.56	654.44	1147.56	1629.97
Standard Deviatic	528.23	504.77	316.67	266.69

with gender or age, with the exception of CG where the older the patient the lower the BDNF at discharge ($p<0.001$) (Table 5).

Chronic LBP represents a major health problem [7-9] and diverse treatments in order to reduce pain and disability are experimented [10]. BDNF, a neuroprotein associated

		Group	
		Control group	HMBG
Schober at admission	Minimum	3.00	3.00
	Maximum	6.00	6.00
	Median	5.00	5.00
	Percentile 25	4.00	4.00
	Percentile 75	5.00	5.00
	Mean	4.68	4.76
	Standard Deviation	.90	.72
Schober at discharge	Minimum	3.00	4.00
	Maximum	6.00	6.00
	Median	5.00	5.00
	Percentile 25	4.00	5.00
	Percentile 75	5.00	5.00
	Mean	4.72	5.12
	Standard Deviation	1.02	.44



Fi. 2. Schober index distribution for patients from the study groups and Box-Plot representation of Schober index quantified scores at admission/discharge for each of the studied groups.

	HMBG	CG	Mann-Whithney U test
Median BDNF at admission (pg/mL)	1211.40 (IQR: 253)	1416.00 (IQR: 1081)	p=0.123 (p>0.05)
Median BDNF at discharge (pg/mL)	1016.80 (IQR: 444)	498.70 (IQR: 714.2)	P<0.001
Wilcoxon test	P<0.001	P<0.001	

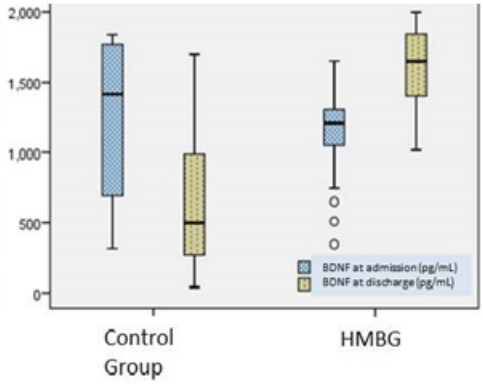


Fig. 3. Statistical evaluation for serum BDNF within each group and between groups both at admission and at discharge and Blox Plot representation of BDNF values of each group at admission and at discharge

HMBG			BDNF at discharge pg/ml	Patient's age
Spearman's r	BDNF at discharge pg/	Correlation Coefficient	1.000	-.012
		Sig. (2-tailed)	.	.953
		N	25	25
	Age	Correlation Coefficient	-.012	1.000
		Sig. (2-tailed)	.953	.
		N	25	25
Control Group			BDNF at discharge pg/ml	Patient's age
Spearman's r	BDNF at discharge pg/	Correlation Coefficient	1.000	-.657**
		Sig. (2-tailed)	.	.000
		N	25	25
	Age	Correlation Coefficient	-.657**	1.000
		Sig. (2-tailed)	.000	.
		N	25	25

**. Correlation is significant at the 0.01 level (2-tailed).

Table 5
AGE CORRELATION WITH BDNF AT DISCHARGE IS SIGNIFICANT ONLY IN CG

with neuropathic pain and mediator of the effects of physical exercise [11, 12], has been extensively studied in recent years, especially regarding the influence of exercise on chronic LBP [13]. The patients in our study had a complex treatment (including hot mud baths) for chronic LBP and we searched if serum levels of BDNF are influenced by this kind of treatment.

Overweight and obesity are reported as risk factors for chronic LBP [6, 9] and analyzing the features of our patients we discovered that less than half of our patients had a normal weight. Obesity itself was found to associate with low levels of BDNF especially in patients with mutations of the gene encoding its receptor - tyrosine kinase receptor type 2 (Ntrk2) [14]. This situation was described especially in children with obesity and psychomotor retardation, as in Biddle-Bardet syndrome [14, 15]. But, despite our expectations, we did not find any significant correlation between BDNF and BMI. This comes in concordance with a similar meta-analysis performed by Sandrini L and colab. in 2018 [16]. We did not find any correlation between serum levels of BDNF and the periodicity of balneal treatment, either.

As women were the majority of our patients, we take into account a future extension of our study, considering the relation between balneal treatment, the levels of serum BDNF and gynecological pathology. A study from Wessels J.M.et al. identified prominent BDNF and Ntrk2 isoforms in the human uterine muscle and endometrium [17]. As magnesium administration induces an increase in BDNF level [17] but also inhibits uterine muscle contraction [18] a possible relation can be found between the two mechanisms involved in uterine relaxation, with possible therapeutic consequences. Another possible future question could be, in this context, if pain decrease could be related also on uterine BDNF-calcium effect, superposed on the known calcium actions on the myometrium [19, 20].

Endometriosis, a benign disease represented by existence of endometrial tissue outside the uterine cavity, is associated with important abdominal pain [21.], sometimes mistaken for LBP. A recent study [22] found that BDNF concentrations in serum and peritoneal fluid were significantly high in women with endometriosis with pain, suggesting that BDNF can play a role in pain's origin. Furthermore, women with endometriosis use physiotherapy and kinesiology in order to reduce pain [23].

VAS evaluation for both groups revealed statistically significant reduction of this parameter and this confirms

that rehabilitation treatment reduces pain [24, 25]. The possible explanation for the greater reduction of VAS score in CG than in HMBG after treatment results from the immediate short term pro-inflammatory effect induced by balneal treatment with sapropelic mud, inflammation that cease in several days from the balneal treatment. So, VAS score is expected to diminish after several days, also. Unfortunately, we did not have the possibility to reassess VAS score in patients from HMBG after discharge. Regarding Schober test we found statistically significant differences only for the patients from the HMBG. This demonstrates the improvement of flexibility of the lumbar spine segment after balneal treatment with hot sapropelic mud, as other studies have revealed [25].

We found that serum BDNF levels are significantly and reversely correlated with patients' age only in CG: the higher the age the lower the BDNF. As our groups did not differ significantly regarding age, perhaps this reduction is induced by balneal treatment without sapropelic mud administration. The result can be biased by the small number of patients included in the study. Statistically significant differences for serum levels of BDNF in both groups, at discharge, were found. Our results revealed a significant reduction in BDNF levels in CG and a statistically significant augmentation of BDNF levels in the HMBG patients. We do not have a clear explanation for the reduction of BDNF levels in CG, we can just suppose that the physical effort during kinetotherapy, even if aerobic, was not strong enough to induce a rise in BDNF level. We compared the results in our CG to other two studies in medical literature. One revealed that only high-intensity locomotor exercise increased the levels of serum BDNF compared to moderate intensity physical exercise in patients with incomplete spinal cord injuries who are not carriers of Val66Met single-nucleotide polymorphism [26] and in another one, in a meta-analysis over 29 studies regarding the effect of exercise training on resting levels of BDNF in peripheral blood, Dinoff A et al. found that aerobic but not resistance training increased blood BDNF levels [27]. In the meantime, the reduction in BDNF serum level in our CG comes in opposition with the results of a study in which aquatic physical therapy twice per week for 5 weeks increased the level of BDNF [3]. In another study, [4] the use of analgesic or antidepressant drugs induced a significant reduction of serum BDNF in old women after an acute episode of LBP. Extrapolating, we can consider that balneal therapy, without sapropelic mud, may have the same effect over serum BDNF levels. We did not find any medical data to compare with the increased levels of serum BDNF in our patients who received balneal treatment with sapropelic mud.

Conclusions

In our study, balneal therapy with Techirghiol sapropelic mud induced a significant elevation of serum BDNF levels, associated with significant pain reduction according to VAS score, and with a significant improvement in lumbar mobility as Schober's test revealed. As we far as we know, up to this moment, there are no studies regarding BDNF serum levels in patients with degenerative chronic LBP under mud therapies, so we think that our study brings significant information in this field and it can represent the starting point for other similar studies over a bigger number of patients.

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CD10, CD34 and Ki67 Immunohistochemical Markers Expression in Endometriosis and Adenomyosis

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Endometriosis is a benign disease represented by existence of endometrial tissue outside the uterine cavity. Considered in the past a type of endometriosis, adenomyosis is, presently, described as a possible different entity, comparative to endometriosis. That is the reason why we decided to study two groups of patients - one with endometriosis and one with adenomyosis - in order to determine if they are one and the same disease. We included all successive patients admitted and surgically treated in the Emergency Clinical Hospital Constanta between 2015-2017, and, after applying the selection criteria, we assessed 61 patients (group 1) diagnosed with endometriosis - ovarian, cervical, caesarian section scar - and 39 patients (group 2) with adenomyosis. We studied all patients in terms of age, parity, lesions' size, admissions' symptoms, chronic symptoms and immunohistochemical markers CD10, CD34, Ki67. We chose there three markers because of their possible relation to endometriosis and because we were unable to find data regarding the comparison of CD34 or Ki67 expression in endometriosis and adenomyosis and because we did not find articles that reported the expression of these three immunohistochemical markers, combined, for either endometriosis or adenomyosis. According to our study, it seems that endometriosis and adenomyosis are different clinically with regard of age and dysmenorrhea, but there was no statistical difference between the studied immunohistochemical biomarkers' expression in samples of patients with endometriosis or adenomyosis.

Keywords: endometriosis, adenomyosis, immunohistochemistry, age, dysmenorrhea

Endometriosis is a benign disease represented by detection of endometrial tissue outside the uterine cavity [1]. It can be found anywhere in the peritoneal cavity: on the ovaries, the fallopian tubes, on the peritoneum, the uterosacral ligaments, the Pouch of Douglas, the rectal-vaginal septum and also in caesarian-section scars, laparoscopy or laparotomy scars, on the bladder, bowels, colon, appendix, and rectum [1].

Adenomyosis, considered in the past a type of endometriosis, is represented by the presence of endometrial tissue in the uterine muscle wall. There are three different types of adenomyosis - focal adenomyosis, focal adenomyoma and diffuse adenomyosis. Some of the theories include tissue trauma or some vaginal injury, which determines inflammation and leads to increased macrophages and cytokines which migrate into the uterine myometrium. Another interesting theory would be extension of deep infiltrating endometriosis from outside into the uterine wall [2].

There is, still, a very important question: endometrioma and adenomyoma are the same entity or are they different?

That is the reason why we decided to study two groups of patients - one with endometriosis (all types) and the other one with adenomyosis. We studied the two groups by comparing the immunohistochemistry (IHC) expression of CD10, CD34 and Ki67, together with the patients' age and admission symptoms.

The IHC marker CD10 is known to be expressed by hematopoietic neoplasms like acute lymphoblastic leukemia and follicular lymphomas, normal endometrial stromal cells and endometrial stromal sarcoma [3].

The IHC marker CD34 represents a transmembrane phosphoglycoprotein, which was first identified on hematopoietic stem and progenitor cells and expresses angiogenesis in tissues [3].

The IHC marker Ki67 is a cellular proliferation marker [3].

We decided to study these three markers considering their relation to endometriosis and to determine if they have the same expression for endometriosis and adenomyosis. Another important reason for this study was that we did not find articles which have studied all the three biomarkers together for endometriosis and adenomyosis.

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Experimental part

Material and methods

In our study we included all successive patients admitted and surgically treated in the Emergency Clinical Hospital Constanta over a period of three years, between 2015-2017. Sixty-one patients (group 1) were diagnosed with endometriosis - ovarian, cervical, caesarian section scar - and 39 patients (group 2) with adenomyosis. We studied all patients in terms of age, parity, lesions' size, admissions' symptoms, chronic symptoms and immunohistochemical markers CD10, CD34, Ki67.

All diagnostics were confirmed by pathology. They were, all, reevaluated by two pathologist and representative samples of each patient were selected for immunohistochemistry. Immunohistochemical tests were performed on four μm-thick sections of formalin-fixed, paraffin-embedded tissue blocks of cases included in the present study. After the epitope retrieval, tissue sections were incubated with the following antibodies from Biocare Medical (ready-to-use): CD10 (56C6 clone), CD34 (QBEnd 10 clone) and Ki67 (SP6 clone). We used 3,3' diaminobenzidine (DAB) as chromogen, with brown staining. Sections were finally counterstained with Mayer's Haematoxylin. A positive membrane immunostain of stromal cell for CD10 was classified in weak, moderate and strong [4]. CD34 immunostain was considered positive if a distinctive brown color was present in the membrane of the endothelial cells or stromal cells. A positive nuclear reaction for Ki67antibody was considered if a brown staining was noticed in more than 5 cells [5].

Experimental data were analyzed with statistical software IBM SPSS Statistics 23. The procedures used were: descriptive statistics (to characterize discrete and continuous variables defined in the data base), Graphics, Nonparametrical statistical tests (Chi-squared test for association, correlation between two category variables, in order to calculate, in specific circumstances, the risk/ chance ratio OR, and Chi-squared test for the comparison of two proportions) [6-9].

Results and discussions

The majority of patients from the group with endometriosis were between 30- 40 years (34 patients - 55.7%), while the respective highest incidence of the patients in the adenomyosis group was in the 40-50 years age interval (30 patients - 76.9%) (table 1). This was not unusual, as adenomyosis and endometriosis are gynecological diseases that are usually common for

Table 1
PATIENTS' AGE IN THE TWO STUDY GROUPS

			Age (years)					Total
			[20-30)	[30-40)	[40-50)	[50-60)	[60-70)	
Group	Endometriosis	Count	7	34	18	1	1	61
		% within Group	11.5%	55.7%	29.5%	1.6%	1.6%	100.0%
	Adenomyosis	Count	0	4	30	4	1	39
		% within Group	0.0%	10.3%	76.9%	10.3%	2.6%	100.0%
Total		Count	7	38	48	5	2	100
		% within Group	7.0%	38.0%	48.0%	5.0%	2.0%	100.0%

Table 2
CD10 IMMUNOHISTOCHEMICAL BIOMARKER'S EXPRESSION IN SAMPLES FROM PATIENTS WITH ENDOMETRIOSIS OR ADENOMYOSIS

			CD10				Total
			Weak positive	Moderate positive	Intense positive	Negative	
Group	Endometriosis	Count	6	52	3	0	61
		% within Group	9.9%	85.2%	4.9%	0.0%	100.0%
	Adenomyosis	Count	8	31	0	0	39
		% within Group	20.5%	79.5%	0.0%	0.0%	100.0%
Total		Count	13	83	3	1	100
		% within Group	13.0%	83.0%	3.0%	1.0%	100.0%

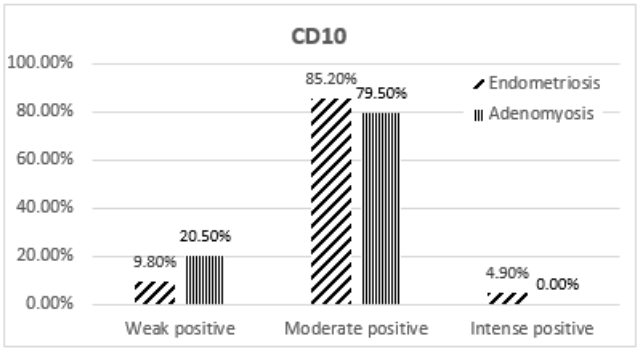

Fig. 1. CD10 immunohistochemical biomarker's expression (%) in samples from patients with endometriosis or adenomyosis

women of reproductive age. The average age for women with adenomyosis is about 40 years [4].

Patients with endometriosis were younger than patients with adenomyosis. There was a statistical difference concerning the age in the two groups ($p < 0.001$, Chi-Square Test). Interestingly, there were 5 patients in menopause in the adenomyosis group and two in the other one.

Thirty patients from group 1 and only 4 from group 2 were infertile. There was a statistical correlation between fertility and each of the two groups. Infertility was more associated to endometriosis than to adenomyosis ($p = 0.001 < \alpha = 0.05$, Chi-Square Test). Our results are concordant with the available data, as approximately 10% of women are diagnosed with endometriosis, but usually it appears in infertile women (40%) [10, 11].

The majority of patients from group 2 (35 patients - 89.7%) had uterine leiomyomas, while only one patient of group 1 was diagnosed with the respective condition.

Dysmenorrhea was mostly associated to group 1, with 42 patients (68.9%), compared with group 2, in which only 10 patients (25.6%) reported it. There was a statistical correlation between the two parameters - group and dysmenorrhea ($p < 0.001 < \alpha = 0.05$, Chi-Square Test). There was a calculated possibility of 6.41 times bigger to find a patient with dysmenorrhea in group 1 than in group 2.

In our study, the biomarker CD10 was positive for all the patients, in both groups, thus confirming the presence of endometrial stromal cells ($p = 0.143 > \alpha = 0.05$, Chi-Square Test) (table 2, fig. 1). It was moderately positive in 52 patients (85.2%) of group 1 and 31 patients (79.5%) of group 2. There was no statistical correlation between the degree of positivity of CD10 and the size of endometriotic or adenomyotic lesions.

Our data were logically relevant and confirm the correctness of the inclusion process, as the immunohistochemical marker CD10 is largely expressed by stromal endometrial cells located outside the uterus. It can certify the diagnosis of endometriosis [4]. It can be also used for women with minimal disease, to confirm the diagnosis [12].

IHC biomarker CD34 was moderately positive in the stromal cells of 18 patients (29.5%) in group 1 and in 7 patients (17.9%) in group 2. It was negative in 43 patients (70.5%) in group 1 and 31 patients (79.5%) in group 2 (table 3, fig. 2). There was no statistical difference between the two groups ($p = 0.213 > \alpha = 0.05$, Chi-Square Test).

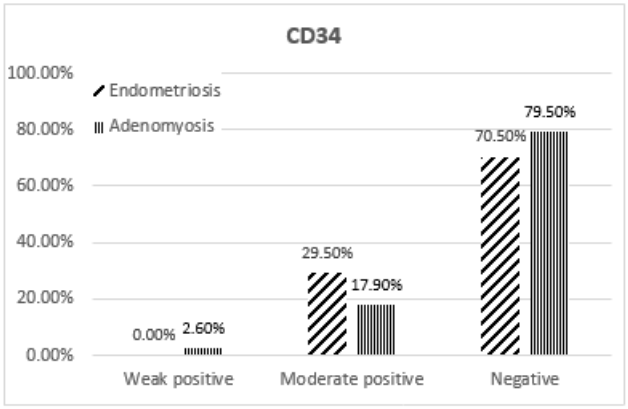

Fig. 2. CD34 immunohistochemical biomarker's expression in samples from patients with endometriosis or adenomyosis

Immunohistochemical marker CD34 is used to identify endothelial cells. Women with endometriosis have an increased cell proliferation in their endometrium. This suggests that the respective endometrium can implant itself and survive in ectopic locations, outside the uterine cavity [13]. Meenakshi M et al. observed a phenomenon of vascular involvement in adenomyosis. When it is widespread, a neoplastic process may be considered. This vascular pattern may sustain a new theory of developing adenomyosis from the cells that are intimately situated to myometrial blood vessels, probably multipotential perivascular cells [14]. The important relation of CD34 with

Table 3

CD34 IMMUNOHISTOCHEMICAL BIOMARKER'S EXPRESSION IN PATIENTS WITH ENDOMETRIOSIS OR ADENOMYOSIS

			CD34			
			Weak positive	Moderate positive	Negative	Total
Group	Endometriosis	Count	0	18	43	61
		% within Group	0.0%	29.5%	70.5%	100.0%
	Adenomyosis	Count	1	7	31	39
		% within Group	2.6%	17.9%	79.5%	100.0%
Total		Count	1	25	74	100
		% within Group	1.0%	25.0%	74.0%	100.0%

			Ki67 Glandular cells			
			Moderate positive	Intense positive	Negative	Total
Group	Endometriosis	Count	19	6	36	61
		% within Group	31.1%	9.8%	59.0%	100.0%
	Adenomyosis	Count	12	1	26	39
		% within Group	30.8%	2.6%	66.7%	100.0%
Total		Count	31	7	62	100
		% within Group	31.0%	7.0%	62.0%	100.0%

Table 4
KI67 IMMUNOHISTOCHEMICAL BIOMARKER'S EXPRESSION IN GLANDULAR CELLS FOR ENDOMETRIOSIS AND ADENOMYOSIS

endometriosis and adenomyosis was the reason why we decided to determine if there is a correlation between the two groups. We could not find in the literature a study comparing the expression of this immunohistochemical marker in-between endometriosis and adenomyosis.

For group 1, IHC biomarker Ki67 (in glandular cells) was positive in 25 patients (40.9%) and negative in 36 patients (59.0%). Patients in group 2 expressed almost the same proportion - 13 patients positive (33.4%) and 26 patients negative (66.7%) (table 4, fig. 3). There was no significative difference, on this regard, between the two groups ($p = 0.213 > \alpha = 0.05$, Chi-Square Tests) (fig. 3).

The biomarker Ki67 in stromal cells was moderate positive in 49 patients (80.3%) and negative in 7 patients (11.5%) in group 1. In group 2 patients, it was moderate positive for 37 patients (94.9%) and negative in one patient (2.6%) (table 5, fig. 4). There was no statistical difference between the two groups for IHC Ki67 in stromal cells ($p = 0.052 > \alpha = 0.05$, Chi-Square Tests) (fig. 4).

Jehn-Hsiahn Yang et al. observed that a high Ki67 index in immunohistochemistry can be predictive for developing adenomyosis [15]. On the other hand, Matsumoto Y et al. studied the bcl-2 gene expression for apoptosis and Ki67 expression as a proliferative marker. They observed that

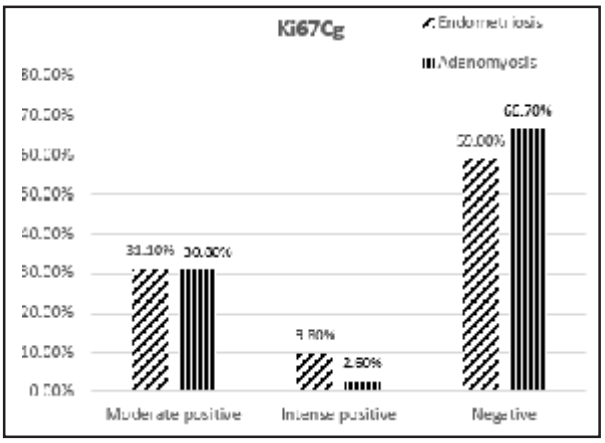

Fig. 3. Ki67 glandular (Ki67 Cg) immunohistochemical biomarker's expression in samples from patients with endometriosis or adenomyosis

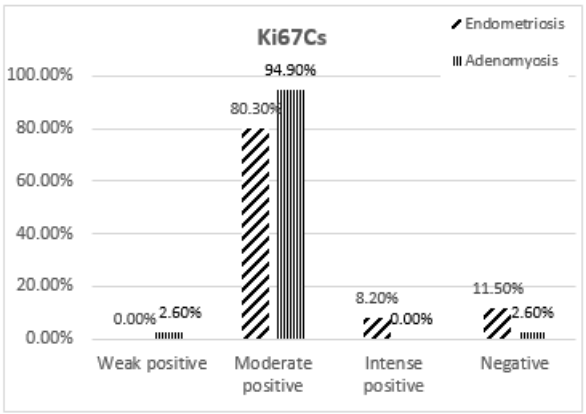

Fig. 4.Ki67 stromal (Ki67 Cs) immunohistochemical biomarker's expression in samples from patients with endometriosis or adenomyosis

Table 5
KI67 IMMUNOHISTOCHEMICAL BIOMARKER'S EXPRESSION IN STROMAL CELLS SAMPLES FROM PATIENTS WITH FOR ENDOMETRIOSIS AND ADENOMYOSIS

			Ki67 Stromal cells				
			Weak positive	Moderate positive	Intense positive	Negative	Total
Group	Endometriosis	Count	0	49	5	7	61
		% within Group	0.0%	80.3%	8.2%	11.5%	100.0%
	Adenomyosis	Count	1	37	0	1	39
		% within Group	2.6%	94.9%	0.0%	2.6%	100.0%
Total		Count	1	86	5	8	100
		% within Group	1.0%	86.0%	5.0%	8.0%	100.0%

Ki67 was expressed by glandular epithelium of ectopic endometrium, without correlation to menstrual phases. In the secretory phase, this immunohistochemical marker was less expressed by eutopic endometrium in functional and basal layers. This is the reason why they concluded that adenomyotic lesions have no origin in the basal endometrium [16]. Because of these data we decided to study the IHC biomarker in both glandular and stromal cells.

As for CD34, we could not find in the literature a study comparing the expression of Ki67 immunohistochemical marker in-between endometriosis and adenomyosis.

Neither could we find data concerning the concomitant expression of CD10, CD34 and Ki67 either for endometriosis nor for adenomyosis. This leads to the logical assertion of the non-available comparison of endometriosis and adenomyosis regarding the bundle CD10, CD34 and Ki67.

Conclusions

Although, in our study, there were significant clinical statistical differences in-between groups with regard to age and dysmenorrhea, immunohistochemical markers CD10, CD34 and Ki67 (glandular or stromal) were similarly expressed in the samples from patients with endometriosis or adenomyosis.

We did not find articles that studied the expression of these three immunohistochemical markers, combined, for either endometriosis or adenomyosis. We were, equally, unable to find data regarding the comparison of CD34 or Ki67 expression in endometriosis or adenomyosis.

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Involvement of Adiponectin in Early Phase of Acute Myocardial Infarction with ST-Segment Elevation (STEMI)

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Adiponectin is secreted by fatty tissue; it has a peptide biochemical structure and among other roles, it seems to inhibit endothelial inflammation. C-reactive protein has an essential role in host defense. Troponin is a protein that is part of cardiac and skeletal muscle contraction. Myocardial infarction is an acute event defined as myocardial cell necrosis, caused by sudden and sustained ischemia. The purpose of our search work was to analyze the connection between various biomarkers over the early phase of acute STEMI: adiponectin, high sensitive-C reactive protein (hs-CRP), high sensitive-Troponin T (hs-Troponin T), triglycerides, total -, LDL- and HDL-cholesterol. Our study included two groups of patients - one group of 30 patients diagnosed with STEMI in the first 12 h after onset, and the second group of 30 patients with unstable angina pectoris, normal hs-Troponin T, and normal findings at coronary angiography computed tomography (CT). Subjects in the STEMI-arm had a higher serum level of hs-CRP, hs-Troponin T, total cholesterol, LDL cholesterol, triglycerides, and decreased levels of HDL-cholesterol and adiponectin than those in the angina-arm. We identified diminished adiponectin plasma concentrations during the first hours of STEMI evolution. We also found out a directly proportional ratio among adiponectin and HDL-cholesterol and an inverse report between this hormone and all other studied biomarkers. Our results may support the anti-inflammatory and anti-atherogenic features of adiponectin.

Keywords: adiponectin, hs-CRP, hs-Troponin T, STEMI

According to its universal definition, an acute myocardial infarction is diagnosed when acute myocardial injury is detected by high and evolutive serum levels of hs-Troponin T (above the 99 percentiles) of the reference scale for a healthy population), together with at least one proof for myocardial ischemia (clinical/ electrocardiography/ imagistic/ coronarography/ autopsy) [1].

Troponin is a protein found in myocardium and skeletal muscle; it is made out of three substructures: Troponin C, T and I; a significant difference is that Troponin C in the skeletal muscle cell contains four calcium-binding sites, while myocardial Troponin C contains only three.

Troponin T and I are released in the blood flow after the loss of cell membrane integrity. Troponin T begins to increase one hour after myocardial infarction outset, gains significant serum levels after 3-4 hours and persists above normal ranges 6-14 days. This prolonged serum persistence is due to the slow release of troponin complex from myocytes and is used for myocardial infarction diagnostics in acute setting and two weeks after [2].

Adiponectin is a plasma cytokine with mainly anti-inflammatory properties. It takes part in glucose metabolism and oxidation of fatty acids [3]. Besides a small amount which is coming from the placenta during pregnancy, it is secreted only by the adult adipose tissue; its plasma level is inversely proportional with adult body adipose mass [4]. Adiponectin secretion has a circadian rhythm, with an early-morning peak and a nocturnal decrease in plasma levels [5].

C reactive protein is the prototype of the acute phase reactants in humans, playing an essential role in host defense. CRP bloodstream levels are low in normal conditions but dramatically increases (about ten thousand fold) in the first hours of all-cause inflammation. CRP is a polypeptide secreted by liver cells as a non-glycosylated monomer, consecutively forming the pentameric structure specific for the pentraxin family of proteins [6].

Cholesterol is found in high amounts in all animal cell membranes, where it takes part in maintaining its structure and also in intercellular signaling [7]. Cholesterol is carried through the bloodstream by the lipoproteins, like LDL and HDL. LDL particles have variable dimensions and density. Clinical trials have shown that small and dense LDL (B model) is a higher risk factor than large and less dense LDL (A model) since smaller particles are more capable of penetrating endothelium. HDL can extract free cholesterol from the cell membrane and attaches it to fatty acids, resulting in cholesterol esters; these are yielded to LDL molecules, in exchange to triglycerides and lipid-soluble vitamins (e.g. vitamin E). In healthy subjects, 30% of plasma cholesterol is transported by HDL [8].

Triglycerides are esters of glycerin with fatty acids, in which all the three hydroxyl groups are esterified. Triglycerides are synthesized by liver cells from carbohydrates. It enters in the structure of VLDL (59%), HDL (3%) and chylomicrons (81-88%), playing a central role in lipid metabolism [9].

The purpose of our search work was to analyze the connection between various biomarkers over the early phase of acute STEMI: adiponectin, high sensitive-C reactive protein (hs-CRP), high sensitive-Troponin T (hs-Troponin T), triglycerides, total -, LDL- and HDL-cholesterol.

Experimental part

Material and method

The active arm of our study enrolled 30 consecutive patients with acute STEMI, admitted in the Cardiology Clinic of Constanta County Hospital in the first 12 h from symptoms onset - during a period of six months (June 2018 - November 2018). The control arm enrolled 30 patients

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All authors with equal scientific contribution and share the first authorship

	Control group	STEMI group	*P* value
Age (mean, years) ± SD	55.4±16.6	58.6±19.3	0.15
Gender (M/F)	18/12	19/11	0.25
Urban/Rural	22/8	21/9	0.34
BMI (kg/m^2)	28.3±6.4	28.7±6.9	0.09
eGFR (ml/min/m2)	62.4±30.8	54.6±24.9	0.04
Systolic blood pressure (mmHg)	154.2±35.4	148.5±34.6	0.03
Diastolic blood pressure (mmHg)	78.6±21.9	76.8±20.8	0.004

Table 1
CHARACTERISTICS OF PATIENTS FROM BOTH STUDY GROUPS

with unstable angina pectoris, who display normal hs-Troponin T and normal findings at coronary angiography CT. Patients in both arms had comparable demographic characteristics (table 1).

Excluding criteria were: chronic inflammatory diseases, active neoplasia, recent (<3 months) trauma or surgery, sepsis or extensive burnings -conditions in which acute phase reactants may be modified; patients who did not agree to participate in our study were also excluded.

The diagnostic of acute STEMI was made on: prolonged acute chest pain, dynamic ST-segment elevation in at least two concordant derivations on ECG, positive markers for myocardial injury (hs-Troponin T) and localized ventricular wall motion anomalies at transthoracic ultrasound examination.

All patients included in our study accomplished anamnesis, physical control and paraclinical tests. Subjects in the STEMI arm underwent all these within the first 12 hours from STEMI onset.

Anamnesis included: age, gender, urban/rural environment, level of education, smoking status, family and personal pathological history, reasons for hospital admission, date and time of symptoms onset and actual disease conditions together with treatment followed by patients until hospitalization.

Physical examination consisted of general status appreciation, BMI calculation and study of respiratory, cardiovascular, digestive, renal apparatus, together with cutaneous/subcutaneous, osteo-articular and central nervous systems.

Paraclinical investigations consisted of:

-ECG: using a 12-derivations Nyhon-Kohden device, in standard conditions (recumbent resting patients); for evidencing of posterior and right ventricle infarction we used extreme left, respectively correct derivations;

-transthoracic echocardiography: using a General Electric Vivid S6 device and making all standard measurements (valves, chambers, systolic and diastolic LV function, segmental wall kinetics, Doppler flows evaluations and great vessels appreciation);

-biochemical blood determinations: using venous blood obtained at the moment of hospital admission; hs-Troponin T was determined using Eclecsys test, adiponectin was determined using the ELISA method and hs-CRP using latex-immunoturbidimetric assay. Triglycerides, total- and HDL-cholesterol plasma concentrations were obtained by the spectrophotometric method, and LDL-cholesterol was obtained by the fourth homogeneous assay. This assay removes non-LDL cholesterol via a selective reaction with cholesterol esterase and cholesterol oxidase, and the resulting peroxide byproduct is eliminated by reaction with catalase.

Normal lipid profile was defined as follows: HDL-cholesterol >40 mg/dL, LDL= cholesterol <100 mg /dL, total cholesterol <200 mg/dL and triglycerides <150 mg/dL.

Statistical analysis was performed with t-test and Pearson correlation coefficient. Continuous variables were summarized as mean ± SD and compared using Student's *t*-test.

Results and discussions

The patients' baseline features are given in table 1, along with the P-values for the univariate analysis.

Of the 30 patients included in the active arm of the study, 20 patients (66%) were diagnosed with dyslipidemia. High-

	Control group *Mean±SD*	STEMI group *Mean±SD*	*P* value
Adiponectin (mg/L	9.05±1.27	6.42±1.41	<0.00001
Total Cholesterol (mg/dL)	200.8±17.69	234.2±38.03	0.005
HDL-Chol (mg/dL)	51±8.32	44±5.43	0.001
LDL-Chol (mg/dL)	104.6±10.17	129.9±32.07	0.009
Triglycerides (mg/dL)	135.8±16.75	181.13±58.56	0.010
hs CRP (mg/L)	0.92±0.29	3.37±2.28	0.0009
hs-Troponine T (pg/mL)	9.29±2.88	1211.15	0.029

Table 2
COMPARISON BETWEEN PATIENTS IN ACTIVE ARM AND CONTROL ARM

	Female Control *Mean±SD*	Female STEMI *Mean±SD*	*P* value
Adiponectin (mg/L)	9.96±1.00	7.24±1.30	0.0005
Total Cholesterol (mg/dL)	192.2±18.61	229.81±39.17	0.031
HDL-Chol (mg/dL)	56±5.47	45.72±5.04	0.001
LDL-Chol (mg/dL)	98.8±4.54	126.81±27.81	0.022
Triglycerides (mg/dL)	129±18.50	161.81±50.56	0.093
hs CRP (mg/L)	0.85±0.26	3.11±1.18	0.0004
hs-Troponine T (pg/mL)	8.94±3.01	725.45	0.026

Table 3
COMPARISON BETWEEN FEMALES-STEMI GROUP AND THE CONTROL GROUP

sensitive troponin T, hs C-reactive protein, adiponectin, triglycerides, total- , HDL- and LDL- cholesterol were analyzed and compared with the group of controls (table 2, 3, 4).

Patients with acute myocardial infarction had increased serum level of triglycerides, total- and LDL- cholesterol, high-sensitive Troponin T and high sensitive C-reactive protein ($P < 0.05$), but decreased plasma levels of adiponectin and HDL- cholesterol ($p < 0.05$) than controls. Concerning equality, these correlations were stronger in females (except triglycerides); in males, these correlations were available only for hs-Troponin T, hs-CRP and adiponectin.

We found a strong inverse ratio between adiponectin and total cholesterol among angina arm females (r=-0.55), and among all patients in agina arm (r=- 0.6) (Table 3), and also a moderate same inverse ratio among male STEMI patients (r=-0.28) (table 4). Adiponectin and HDL-cholesterol exhibited a powerful positive relation among females in the angina arm (r=0.56), and a moderate positive one for the male STEMI arm (r=0.42), total angina arm (r=0.42), and all subjects (r=0.39). Important inverse ratio was found between adiponectin and LDL cholesterol in the male angina arm (r=-0.77), total angina arm (r=-0.64), and also a slight inverse ratio in the male STEMI arm (r=- 0.49), and all subjects (r=-0.36). We found a solid inverse ratio between adiponectin and triglycerides in female angina arm (r=-0.53), in female STEMI arm (r=-0.58), and a moderate inverse one for total angina arm (r=-0.41), and in all subjects (r=-0.38). Strong negative correlation with high sensitive C-reactive protein was found in the male control group (r=-0.66), in male STEMI patients (r=-0.89), and all patients (r=-0.64). Strong negative correlation with high sensitive troponin T was recorded for female STEMI subjects (r=-0.57), male STEMI subjects (r=-0.86), and all STEMI subjects (r=-0.74) (table 5).

There have been numerous in vitro studies, as well as studies in animal models and clinical studies, which highlighted the link between circulating adiponectin levels and cardiovascular diseases.

In vitro studies:

We must mention the most popular in vitro studies, as follows:

-Ouchi et al. showed that adiponectin inhibits the shift of macrophages into foam cells, may be acting as a mediator for this conversion, and thus making a connection between atherosclerosis and endothelial inflammation [10];

-another study showed that adiponectin might play an important role in adjusting the effects of insulin; subjects with type 2 diabetes mellitus recorded low adiponectin concentrations, which may concur to tissue insulin resistance [11];

-Matsuda et al. conclude that adiponectin exerts an inhibitory outcome on the HB-EGF (heparin-binding epidermal growth factor-like) expression in endothelial cells treated with TNFα and down-regulates the migration of activated smooth muscle cells [12];

-Yokota underlined the inhibitory effect of adiponectin on the proliferation of myelomonocytic cell line and mature macrophage functions such as TNFα production and phagocytosis [13];

-according to Chen, adiponectin inhibits the synthesis of nitric oxide in endothelial cells [14], and Arita et al concluded that adiponectin has a negative effect on the multiplication and migration of smooth muscle cells set by platelet-derived growth factor [15].

All these experimental studies have concluded that adiponectin has direct anti-atherogenic and anti-inflammatory effects on the endothelium, interfering all stages of plaque formation.

	Male Control *Mean±SD*	**Male STEMI** *Mean±SD*	***P* value**
Adiponectine (mg/L)	8.13±0.74	5.95±1.27	0.0007
Total Cholesterol (mg/dL)	209.4±13.16	236.73±38.20	0.067
HDL-Chol (mg/dL)	46±7.96	43±5.53	0.167
LDL-Chol (mg/dL)	110.4±11.32	131.68±34.90	0.099
Triglycerides (mg/dL)	142.6±13.16	178.89±71.78	0.139
hs-CRP (mg/L)	1.002±0.33	3.54±2.74	0.027
hs-Troponine T (pg/dL)	9.64±3.04	1471.29	0.040

Table 4
COMPARISON BETWEEN MALES- STEMI GROUP AND THE CONTROL GROUP

Table 5
ADIPONECTIN AND LIPID PROFILE/ HS-CRP/ HS-TROPONIN T' RELATIONSHIP AMONG THE STUDIED GROUPS

	Female Control	**Male Control**	**Female STEMI**	**Male STEMI**	**Total Control**	**Total STEMI**
Total Cholesterol (mg/dL)	-0.55	-0.02	0.02	-0.28	-0.6	-0.19
HDL-Chol (mg/dL)	0.56	-0.75	0.14	0.42	0.42	0.39
LDL-Chol (mg/dL)	0.22	-0.77	-0.12	-0.49	-0.64	-0.36
Triglycerides (mg/dL)	-0.53	0.57	-0.58	-0.3	-0.41	-0.38
hs-CRP (mg/L)	- 0.05	-0.66	0.04	-0.89	-0.24	-0.64
hs-Troponine T (pg/dL)	-0.23	-0.11	-0.57	-0.86	-0.08	-0.74

Studies in animal models

-Kubota et al. used adiponectin knockout animals and showed that neointimal proliferation in response to injury is accelerated. [16]

-Okamoto et al. observed, by the use of immuno-histochemical techniques in mice, the accumulation of the adiponectin in the mechanically destroyed carotid artery and afterward demonstrated that the administration of adiponectin to apolipoprotein E knockout mice significantly reduce the progression of atherosclerotic lesions [17, 18].

Clinical studies

-Jansson et al have reported the inverse correlation between adiponectin plasma concentration and endothelial dysfunction [19];

-Hotta et al emphasized that in subjects associating coronary artery disease and type 2 diabetes mellitus plasma adiponectin was significantly decreased [20];

-Zoccali et al followed a group of patients with end-stage renal disease and observed that low adiponectin was an independent predictor of cardiovascular events [21];

-Adamczak et al noticed decreased circulating adiponectin in subjects with essential hypertension compared with normotensive subjects [22];

-Okamoto et al have shown that adiponectin is bound to collagen type I , III and V found in the vessels' frame, particularly in the event of an injury at this level, taking part in the repair process [17];

-Margaritis et al emphasized that adiponectin raises nitric oxide (NO) production by PI3 kinase/Akt-mediated phosphorylation and activation of eNOS [23];

-Cheng et al pointed out that disturbances in adiponectin and insulin signaling paths increase the risk of metabolic and vascular disorders [24].

Our study revealed an opposite relation between adiponectin and triglycerides, total- and LDL-cholesterol, and a linear relation between adiponectin and HDL-cholesterol, disclosing adiponectin as a protective factor against atherosclerosis. The opposite relationship between adiponectin and high-sensitive troponin T may denote an increased accumulation of adiponectin in the injured myocardial region, with a possible tisular regenerating role, as other studies suggest [25, 26].

Concordant to literature data [27, 28], our study pointed out that an important acute reaction of CRP accompanies myocardial infarction. Other studies concluded that CRP and cardiovascular diseases are complement-related: CRP promotes endothelial expression of adhesion molecules, in a genotype-dependent manner [29, 30]. These facts sustain the theory that CRP has a main role in the atherosclerosis inflammatory pathway [31].

A recent update [32] emphasized that hypoadiponectinemia correlates with an increased cardiovascular risk given the fact that low adiponectinemia promotes insulin resistance [33] and that serum adiponectin levels grew concordant with insulin resistance's improvement after renin-angiotensin system blockade [34].

A large trial [35] has shown that high adiponectinemia was independently correlated with augmented risk of death by all-cause (including cardio-vascular) in subjects associating type 2 diabetes mellitus and recent acute coronary syndrome; the same correlation was found in an elderly cohort [36] and in non-diabetic patients discharged after an acute myocardial infarction [37,38].

However, the conflicting results were recently analyzed [39], but sustainable explanations – concerning adiponectin tissue resistance, genetic variants, sex-related effects or simply altered results by unexpected confounders - were not yet offered for the *adiponectin paradox* on cardiovascular and all-cause mortality.[40]

Limitations

Nowadays, an important biochemical issue is the fact that all adiponectin isoforms are recognized by commercial assay kits measuring the total adiponectin concentrations [41]. The ELISA technique used in our study detects the globular and full-length isoforms. It is well known right now that each adiponectin isoform is playing a different biological role.[42-44] The proportion of isoform expression is altered in various physiological and pathological conditions. For example, to predict risk mortality in heart failure populations, total adiponectin is usually recommended [42,43].

Newly developed adiponectin assays can measure each isoform separately, so further research is needed to establish the role of isoforms in different pathologies [45].

Another shortcoming is the reduced number of patients included in this study. Larger studies are needed to further refine the relationship among adiponectin and other biomarkers in the acute phase of STEMI.

Conclusions

In our study we revealed an inverse ratio between hs-CRP and adiponectin during the first hours of STEMI evolution, which may support the anti-inflammatory and anti-atherogenic role of adiponectin. We identified diminished adiponectin plasma concentrations during the first hours of STEMI evolution.On the other hand, the study revealed a directly proportional ratio among adiponectin and HDL-cholesterol and an inverse report between this hormone and all other studied biomarkers.

Low circulating adiponectin concentrations in the setting of acute STEMI, its inverse correlation with LDL-cholesterol, hs-troponin T, hs-CRP together with its positive correlation with HDL- cholesterol, gave us arguments to assume that this adipokine may be a protective cardiovascular factor, but the result is difficult to be interpreted.

Abreviation

BMI=body mass index
CRP= C-reactive protein
CT= computed tomography
ECG= electrocardiography
eGFR= estimated glomerular filtration rate
eNOS= endothelial nitric oxide synthase 3
HB-EGF= heparin binding epidermal growth factor-like
HDL-cholesterol, HDL-chol= high density lipoproteins cholesterol
hs Troponin T= high sensitive Troponin T
hs-CRP= high sensitive C reactive-protein
LDL-cholesterol, LDL-chol= low density lipoprotein cholesterol
NO= nitric oxide
STEMI= myocardial infarction with ST-segment elevation
TNFα=tumor necrosis factor α
VLDL= very low density lipoproteins

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CASE REPORT

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Ovarian teratomas in a patient with Bardet–Biedl syndrome, a rare association

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Abstract

Bardet–Biedl syndrome (BBS) represents a rare ciliopathy recessive autosomal inherited. The main clinical features are retinal dystrophy, postaxial polydactyly, obesity, different degrees of cognitive deficit, renal impairment, hypogonadism and genital malformations. The genetic explanation consists in BBS genes mutations, which encode modified proteins, altering the function of the immotile cilia. As a multitude of BBS genes mutations were described, the phenotypic aspect of these disorders varies according to that. We present the case of a 22 years old female patient, known with BBS since the age of 11 and which was diagnosed and operated for bilateral ovarian dermoid cysts, at the age of 21. We did not find a similar case in literature, regarding the association between the two disorders. We consider that our case points towards the importance of periodic imagistic evaluations [magnetic resonance imaging (MRI), computed tomography (CT) or ultrasound] of these patients, not only clinical and biological. Usually, the moment they are diagnosed with hypogonadism or genital malformations (in childhood or adolescence), the genital evaluation is neglected thereafter. We also consider that our therapeutic approach can be helpful in other similar clinical situations. Another important conclusion is represented by the importance of genetic counseling of the relatives of a BBS patient, unfortunately insufficiently provided in our region.

Keywords: Bardet–Biedl syndrome, retinal dystrophy, hypogonadism, dermoid cysts, ovaries.

Introduction

Bardet–Biedl syndrome (BBS) represents a rare genetic disorder with autosomal recessive transmission. Its prevalence varies, according to *National Library of Medicine* (US)–*Genetics Home Reference*, from Bedouin population in Kuwait (1:13 500) and Canada (1:17 500) to Europe (1:160 000) and Japan (only 38 possible cases in 2011) [1, 2]. Even if in the beginning, it was named Laurence–Moon–Bardet–Biedl syndrome and thereafter separated into two entities: Laurence–Moon and Bardet–Biedl, now the consecrated term is BBS.

The major clinical expression of BBS is represented by retinal dystrophy, post-axial polydactyly, obesity, different degrees of cognitive deficit, renal impairment and hypogonadism. Many other disturbances can occur, like genital malformations, facial dysmorphysm, disorders of posture and gate, etc. [3]. They are not all found in the same patient and they do not have the same expression in all the patients, probably because at least 20 different genes (called BBS genes) show mutations in different combinations [4, 5].

Cilia are cellular structures with tubular aspect found on the apical surface of the majority of the cells and they are involved in cell movement (motile cilia) and in chemical signaling pathways (immotile cilia). Cilia are also necessary for the perception, acting like sensory organelle.

The mutated BBS genes encode modified proteins, which in turn alter the function of immotile cilia. Immotile cilia are clinically translated into retinitis pigmentosa, renal, hepatic and pancreatic cysts, polydactyly, learning difficulties [6].

The rate of survival of these patients rarely exceeds 60 years old but studies are limited in this field. Some of them are depicting a median survival of 63 years [7], others are presuming less than that [3].

Dermoid cysts represent germ cell tumors containing different tissues developed from one or all three germinative layers, most frequently from the ectodermic layer. Because of their germinal origin, they are frequently found in the gonads, ovaries in the first place. The dermoid cysts and teratomas are in the majority of cases the same entity. Teratomas can be mature, well differentiated and benign, usually with cystic appearance, or immature, mainly solid and malignant. Their treatment is surgical, with significant possible complications and morbidity [8].

We present for the first time, up to our knowledge, a case of BBS associated with ovarian teratomas. We found other cases of teratomas associated with Bardet–Biedl syndrome but with different locations and different treatment options [9].

Patient's legal tutor written approval was obtained in order to publish the case.

Case presentation

A 20-year-old patient came in consultation for hypermenorrhea, menorrhagia, irregular menses and pallor in the II[nd] Medical Department of the Railways Hospital, Constanţa, Romania, in 2014.

Her familial history revealed that she had a younger sibling who was diagnosed with BBS (he died at the age of eight, due to chronic renal failure) and that she has a cousin with BBS, also.

Until the age of 11 years old, she was not evaluated for BBS even if she was born with bilateral lower limbs polydactyly (surgically removed at one year of age), and even if her younger brother was already diagnosed with this disorder, before she turned 11.

She was diagnosed with BBS at 11 years old, when she was endocrinological evaluated for obesity [height 160 cm and weight 77 kg; body mass index (BMI) 30 kg/m^2], visual impairment, brachydactyly, mild learning disability. She started to lose her night vision at the age of 9 and now she is able to detect only light. At that time, the check-up revealed dyslipidemia (serum cholesterol 265 mg/dL, triglycerides 483 mg/dL), subclinical hypothyroidism [thyroid-stimulating hormone (TSH) 6.8 µIU/mL, without autoimmunity], altered oral glucose tolerance test (OGTT) but with a normal value of HbA1c (glycosylated hemoglobin) and mild spasticity of the lower limbs (Table 1). Diet and 50 µg of L-Thyroxine/day were recommended as well as further investigations in a specialized service.

Table 1 – ***Patient's biological and humoral parameters over time***

Age [years]	***11***	***12***	***16***	***20***	***21***	***22***
L-Thyroxine treatment [µg/day]	***Without treatment***	***50 µg L-Thyroxine***	***50 µg of L-Thyroxine***	***50 µg of L-Thyroxine***	***75 µg of L-Thyroxine***	***90 µg of L-Thyroxine***
Height [cm]	160	164	168	170	170	170
Weight [kg]	**77**	**85**	**84**	**76**	**75**	**75**
BMI [kg/m^2]	**30**	**31.6**	**28.8**	**26.3**	**26**	**26**
Glycemia à jeun (60–99 mg/dL)	**123**	74	86	84	87	101
OGTT [mg/dL]	**201** at 1 h **182** at 3 h **155** at 4 h	106 at 1 h 99 at 2 h 100 at 3 h	–	–	–	–
Creatinine (0.6–1.2 mg/dL)	1.21	1.1	0.9	1.03	1.02	0.81
Urea (<43 mg/dL)	25.3	22.5	23	38	36	35
Total cholesterol (<200 mg/dL)	**265**	191	220	**263**	**254**	**301**
Triglycerides (<150 mg/dL)	**483**	**215**	200	95	**160**	118
FSH (children <5.1 mIU/mL; adult women 3.5–12.5 mIU/mL)	0.41	–	–	5.2	5.6	–
LH (children 0.2–1.4 mIU/mL; adults 2–12.6 mIU/mL)	0.19	–	–	**20.6**	**24.1**	–
Progesterone – luteal phase (5.3–86 nmol/L)	–	–	–	**0.67**	**0.59**	**0.57**
Estradiol – follicular phase (46–607 pmol/L)	–	–	52.7	180.1	230.3	–
TSH (0.51–4.3 µIU/mL)	**6.8**	1.6	2.53	**6.99**	**5.45**	1.1
ATPO (<50 IU/mL)	10.6	–	–	16.5	–	–
AbTGB (<115 IU/mL)	–	–	–	60.3	–	–
FT4 (0.8–2.02 ng/dL)	**0.6**	1.4	–	0.8	1.2	–
Prolactin (72–511 µIU/mL)	–	–	183	173	–	251.9
17-OH-KT (3–12 mg/24 h)	–	4.02	–	–	–	–
17-KT (3.3–11.5 mg/24 h)	–	7.8	–	–	–	–

BMI: Body mass index; OGTT: Oral glucose tolerance test; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; TSH: Thyroid stimulating hormone; ATPO: Anti-thyroid peroxidase antibodies; AbTGB: Anti-thyroglobulin antibodies; FT4: Free tetraiodothyronine; 17-OH-KT: 17-OH-ketosteroids; 17-KT: 17-Ketosteroids. Abnormal data are in bold.

She repeated a clinical and lab check-up, one year later (at the age of 12). She had, while treated, BMI 32 kg/m^2, normal OGTT, hypertriglyceridemia (215 mg/dL), normal TSH (1.6 µIU/mL). Hypothalamic-hypophyseal computed tomography (CT) scan revealed empty sella with frontal cortical atrophy. Gonadal and suprarenal functions were evaluated at that moment and found within normal limits (Table 1). Abdominal CT scan did not reveal any renal malformations. Ophthalmological evaluation established the diagnosis of atypical retinitis pigmentosa. Diet, L-Thyroxine and periodic evaluations were recommended.

Menarche occurred at 14 years old.

At 16 years old, her biological parameters confirmed the persistence of dyslipidemia (serum cholesterol 220 mg/dL and triglycerides 200 mg/dL) and the normalized thyroid function (TSH 2.53 µIU/mL, under treatment). Renal ultrasound and functional evaluation where within normal limits, too.

Until the age of 20, she was not evaluated any further – until menstrual disorders occurred and the patient presented in our Department. She kept, all the same, a healthy hypocaloric, hypolipidemic diet, rich in vegetables, fruits and lean meat.

Physical examination revealed facial dysmorphism with flat nose bridge and with anteverted nares, downward slanting palpebral fissures, high arched palate, small teeth and enamel hypoplasia, normal height (170 cm) and overweight (76 kg). She has brachydactyly and brachymetacarpia, especially in the 4th and 5th fingers, multiple skin nevi on the thorax and abdomen, micromastia, normal sexual body hair. She has mild ataxia, lateral nystagmus, and the mild spasticity of the lower limbs persists (Figure 1).

During her endocrinological work-up, we found low levels of progesterone in luteal phase, a luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio of almost 4 in early follicular phase, subclinical hypothyroidism (under treatment with 50 μg of L-Thyroxine/day) and a cystic aspect of the ovaries at trans-abdominal ultrasound (virgo patient who refused ultrasound examination with intra-rectal probe). L-Thyroxine was increased gradually up to 90 μg/day and TSH values were normalized. Dydrogesterone 20 mg/day 10 days per menstrual cycle was started. Under this treatment, menses became regular, with a five days duration and normal flow, occurring at 28–29 days. After nine months of treatment, the patient stopped her progestin treatment and bradymenorrhea occurred. The LH/FSH ratio was 4.3 and the ultrasound revealed that cystic ovarian aspect maintained but they seemed to modify their inner aspect (hyperechoic areas, even calcifications). She was HIV (human immunodeficiency virus) negative. Pelvic magnetic resonance imaging (MRI) was performed and revealed polycystic ovaries and the suspicion of bilateral ovarian dermoid cysts also, of 2.1 cm and 7.2 mm in diameter (Figures 2 and 3).

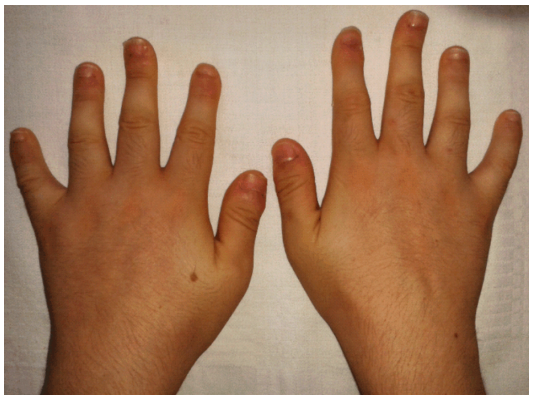

Figure 1 – ***Brachydactyly of the IVth and Vth finger.***

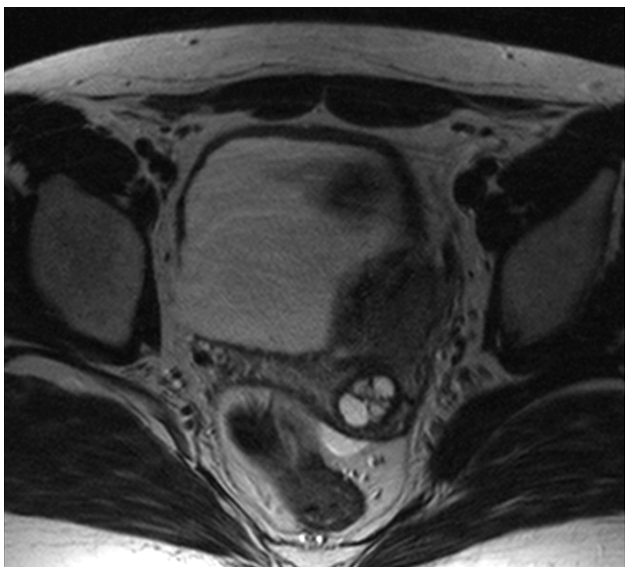

Figure 2 – ***Pelvic MRI: left ovary dermoid cyst.***

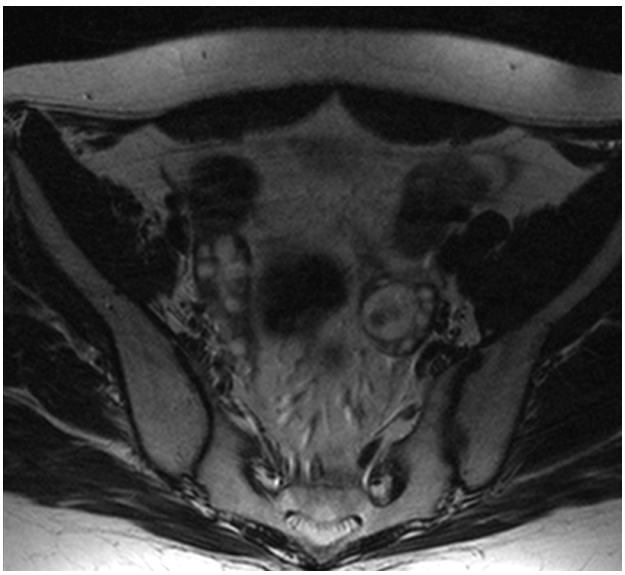

Figure 3 – ***Pelvic MRI: bilateral polycystic aspect of the ovaries.***

Laparoscopic surgery was performed in the Ist Gynecology Department of the Emergency County Hospital of Constanţa and two ovarian cysts, one on each side and one paraovarian cyst on the left were removed (Figures 4–7).

Histopathological examination was performed in the Department of Pathology of the Emergency County Hospital of Constanţa and the presence of mature bilateral teratoma along with one simple cyst was confirmed. Patient's family asked for a second opinion so, two blocks were analyzed in the Department of Pathology of the "St. Pantelimon" Emergency Hospital, Bucharest, Romania. The results were the same: fragments of ovarian tissue along with sebaceous glands, pilous follicles, and cystic areas with parakeratotic pluristratified pavement epithelium; cellular detritus, squames, all confirming bilateral ovarian dermoid cysts (Figures 8–10).

Progestin and L-Thyroxine treatment were continued.

One year after, menses are regular, and ultrasound of the ovaries reveals simple cystic aspect. Renal function is normal and a mild dyslipidemia persists.

Discussion

Because the time between the onset of symptoms and signs and diagnosis is quite long (up to eight years in some studies) [7], the majority of BBS patients looking healthy at birth unless they were born with polydactyly, or because other symptoms of BBS gradually appear during or after the first decade of life, an attempt was made by Beales *et al.*, in 1999, to modify the existing diagnostic criteria of BBS, in order to help establishing an earlier diagnosis, especially in children [3].

These criteria are divided into six primary: rod-cone dystrophy, polydactyly, obesity, renal defects, genital abnormalities and learning difficulties and secondary features: developmental delay, speech deficit, brachydactyly or syndactyly, dental defects, ataxia or poor coordination, olfactory deficit, diabetes mellitus, and congenital heart disease. Four primary or three primary and two secondary criteria are necessary to establish the diagnosis of BBS [6, 7].

Even if BBS gene testing was not available for us, the clinical criteria of diagnosis are fulfilled by our patient.

These are: macular dystrophy, polydactyly, obesity, learning difficulties – as major criteria and brachydactyly, and slight ataxia – as a secondary criteria. She has luteal insufficiency also, sustained by her FSH, LH and progesterone levels, as well as by the good menstrual response at progestins.

The age at diagnostic, 11 years, was older than the median of 8 found by the authors cited before [7], even if important clues were available: polydactyly at birth and a sibling diagnosed with BBS when our patient was 7 years old. We think that an earlier diagnosis might have improved the measures taken especially in reducing dyslipidemia and obesity as cardiovascular risk factors.

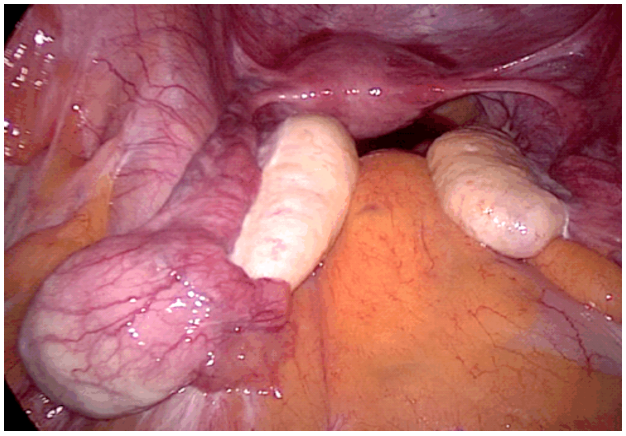

Figure 4 – ***Laparoscopic view showing normal (rather smaller) uterus, normal tubes, a para-ovarian cyst and slightly enlarged, elongated, ovaries.***

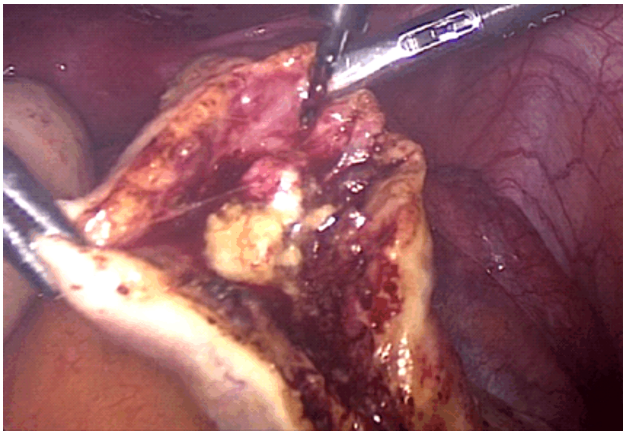

Figure 5 – ***Laparoscopic view showing fatty-sebaceous tissue inside the right ovary, during cystectomy.***

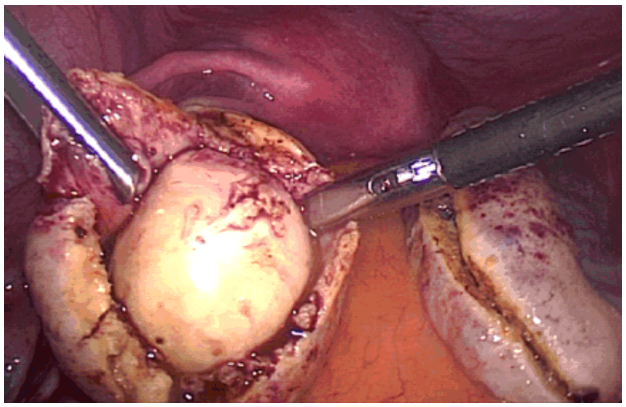

Figure 6 – ***Laparoscopic view showing the dermoid cyst within the left ovary, during the cystectomy.***

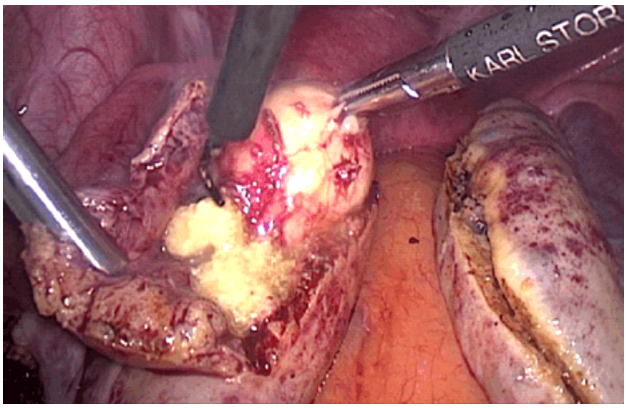

Figure 7 – ***Laparoscopic view showing expulsion of sebaceous-fatty tissue during the left ovarian cystectomy.***

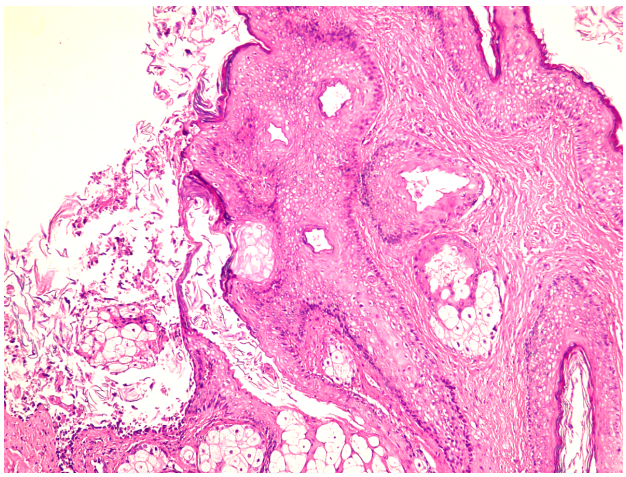

Figure 8 – ***Left ovarian dermoid cyst – pluristratified pavement epithelium with keratinization, sebaceous glands and pilous follicles. HE staining, ×200.***

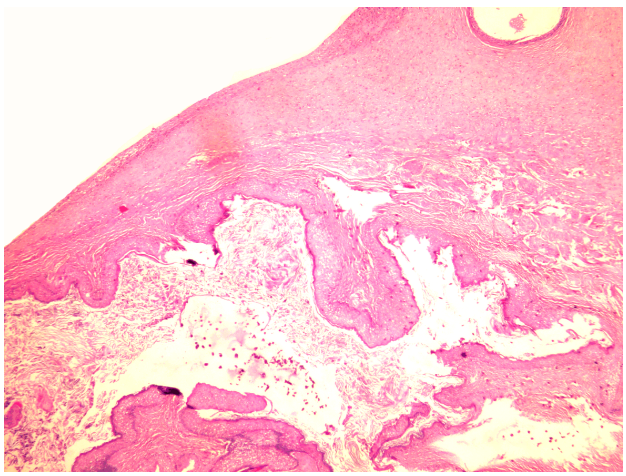

Figure 9 – ***Left ovary image with follicular cyst (upper right) and keritinized pluristratified epithelium and keratin squames inside the mature dermoid cyst. HE staining, ×40.***

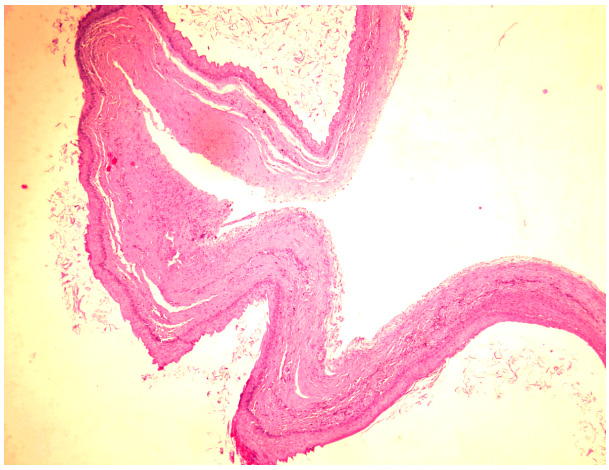

Figure 10 – ***Right ovarian dermoid cyst – ovarian tissue surrounded by keratotic pavement pluristratified epithelium which forms cystic areas with keratin content. HE staining, ×40.***

Anyway, we think that our patient has an attenuated form of the disease as her retinal dystrophy did not evolve in the last 3–4 years and she did not suffer any other worsening of her already known disorders. However, in order to sustain this hypothesis, a genetic test would be highly illustrative.

Available data is in favor of the frequent occurrence of renal impairment associated with BBS. This consists in renal cysts, renal scarring, hydronephrosis, fetal lobulated aspect, renal dysfunction up to renal failure, renal carcinoma, etc. [7, 10]. There are also proofs that renal malformations and renal carcinoma are frequent among the BBS-free relatives of BBS patients – and the explanation can be heterozygosity for BBS genes [7]. Sure, prenatal renal malformations are equally not relevant for this case [11, 12]. There is a study which report that first-degree relatives have no predisposition to metabolic and renal disorders [6] but the small number of subjects included in the study can be a bias. These findings have important implications for the care of BBS patients and their BBS-free relatives. This was the case of our patient's sibling, also. Fortunately, until now, our patient renal function is normal.

We did not find in the literature another report of a case of BBS associated with ovarian mature teratoma. We do not know if this is an occasional association or not. We consider, therefore, that periodic total abdominal and thoracic investigation (ultrasound, MRI), not only renal or cardiac, is necessary in order to detect tumor occurrence.

We choose to operate her for these tumors because of the possible future complications: atypical rapid growth (as they were not detected in the previous ultrasound examinations, even abdominal CT scans), torsion, even carcinomatous transformation [13]. HIV testing was considered appropriate as she lives in a region with a previous relatively high incidence of HIV infection [14].

Men with BBS are usually infertile [15] but a case of reversible hypogonadotrophic hypogonadism in a male who developed spontaneous reversal of hypogonadism in adulthood was reported [16]. Women have irregular menses and they may develop both gonadal failure and central hypogonadism [17]. There are rare cases reported with preserved fertility, probably those with incomplete gene penetration [7]. We assumed that an early menopause could occur due to ovarian surgery associated with usual premature ovarian failure found in these patients [7], but, fortunately, until this moment it did not.

For the moment, treatment options for patients with BBS are mostly symptomatic. However, gene therapy can be the option of the future. There are already some promising experiments in animals, like topical subretinal injection of BBS-containing adenovirus, which delivered the missing BBS gene and rescued rhodopsin mislocalization and preserved the function of the eyes in experimental mice [18, 19]. Another such experiment is the administration of a melanocortin receptor agonist that attenuated obesity in BBS knockout mice, probably activating leptin receptor signaling [20].

In May 2010, an interesting case was published in "Clinical Genetics" by Genuis & Lobo, describing a 21 months old girl with BBS, who underwent testing for biochemical deficiencies and in whom nutritional status correction was undertaken. Surprisingly, patient's signs and symptoms (deteriorated vision, obesity, behavior and mood disorders) subsequently resolved over the course of several months and she maintained the normal status until the publication time – that was 7 years old [21].

In the light of this paper, it is possible that the setback of our patient's disorders during the last two years (with the exception of the teratoma occurrence that we do not know if it is related to BBS) could be due to the change in her diet.

We consider our case presentation important not only because of the unique association of diseases but also because of the problems issued by the treatment of this patient. Apart from the possible surgical accidents and incidents, the risk of, even partial, ovarian removal, might have induced a premature menopause in a 21 years old patient with ovarian failure and under the risk of central hypogonadism, with all the emerging consequences at metabolic, osseous, cardiovascular and psychic implications.

We also emphasize the importance of genetic counseling, especially in families in which cases of Bardet–Biedl syndrome were described, as our case. In case of IVF (*in vitro* fertilization) along with other ovarian parameters' evaluation [22], pre-implantation genetic diagnostics are available in families where the genetic mutation is known [23].

Conclusions

It is, up to our knowledge, the first time that an association BBS–ovarian teratomas is reported. The clinical implications lead us to consider that periodic total abdominal and thoracic investigation (ultrasound, MRI), not only renal or cardiac, is necessary in order to detect tumor occurrence. Due to possible complications, surgical laparoscopic removal of the teratomas is the best treatment option, with the help of an experienced surgeon. Genetic counseling is of major importance in BBS, in order to reduce the disease's burden and take appropriate preventive actions.

Conflict of interests

The authors declare that they have no conflict of interests.

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Author contribution

Irina Tica and Oana-Sorina Tica equally contributed to this article.

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CASE REPORT

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Giant abdominal tumor – would you think adrenal?

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Abstract

Adrenal cavernomas are rare benign tumors, and those of giant dimensions are exceptional. Usually, they are symptomless or they induce symptoms and signs due to compression over nearby organs. We present the case of a 68-year-old woman, who complained of abdominal enlargement and abdominal pain in the left part of the abdomen. Imagistic investigations (native and contrast abdominal computed tomography) revealed an inhomogeneous retroperitoneal mass of 210/182/200 mm, containing calcifications. Laboratory findings were not relevant, just a slight and non-significant elevation of carcinoembryonic antigen and a slight elevation of C-reactive protein. Diagnosis of cancer of undetermined origin was considered, and surgery was performed. During surgery, a giant encapsulated inhomogeneous tumor of 330 mm, with cystic areas, was removed, without assessing the origin. Primary or secondary tumors (metastasis from breast, intestinal, lung, renal or skin cancer) were taken into account. Only histopathology and immunohistochemistry revealed the diagnosis of adrenal cavernoma. Until this moment, we found only one published article in the medical literature with similar dimensions of an adrenal cavernoma as in our case. Even if rare, hemangioma of the adrenal gland must be considered during the differential diagnosis of an adrenal tumor.

Keywords: tumor, adrenal, hemangioma, giant cavernoma.

Introduction

Cavernomas are benign tumors consisting of blood vessels lined by normal endothelial cells, which can confluence and form large cystic areas or even areas of thrombosis. They can occur in any area with blood vessels, most frequently in brain, eyes, liver [1] but their occurrence in adrenal gland is rare. We have found very few (less than 100) cases of adrenal cavernoma that have been described in the medical literature since 1955, when the first case was reported by Johnson & Jeppesen. Adrenal cavernomas are rare, with an incidence of 0.01% of the adrenal tumors [2], and they infrequently reach giant dimensions. In the vast majority of cases, they measure up to 10–15 cm [3–9].

Until this moment, we found only one published article in the medical literature with similar dimensions of an adrenal cavernoma as in our case [9].

The rarity of this tumor, as well as the important problems of differential diagnosis regarding the possible malignancy, the surgical diagnostic and therapeutic difficulties make this case presentation interesting and useful to clinicians.

Case presentation

We present the case of a 68-year-old woman, who was admitted in the II[nd] Medical Department of Emergency County Hospital, Constanţa, Romania, in August 2018, for the evaluation of an enlargement of the abdomen and for pain, especially in the epigastric area.

Patient's written consent to publish her medical data was obtained, as well as the approval of the Hospital Ethics Committee.

The patient had noticed progressive enlargement of the abdomen during the previous year, and, in the last two months, she had also complained of aggravated constipation.

An ultrasound (US) examination was performed before admission into the Hospital and a giant inhomogeneous, mainly hypoechoic tumoral mass, with calcifications, vascularized septa and cystic areas, was described. Because the tumor occupied the entire left hemiabdomen, dimensions, origin or relations to the nearby organs were impossible to be assessed by US examination.

From her medical history, we also report type 2 diabetes mellitus (treated with oral antidiabetic drugs), stage 3

essential hypertension (treated with diuretics), subtotal thyroidectomy (treated with 132 μg of Levothyroxine per day), osteoporosis and cholecystectomy for biliary lithiasis.

Physical examination revealed an obese patient (body mass index 35.9 kg/m^2), with an enlarged abdomen and with a palpable tumoral mass in the entire left half of the abdomen, with no other pathological signs.

The performed native and contrast abdominal computed tomography (CT) indicated cortical renal cysts, with sizes up to 52/42 mm, and a macronodular, inhomogeneous, retroperitoneal mass, with the presence of infracentrimetric calcifications, mainly peripheral contrast enhanced, with axial diameter of 210/182 mm and a cranio-caudal one of 200 mm, pushing rightwards the aorta and the left kidney (Figure 1).

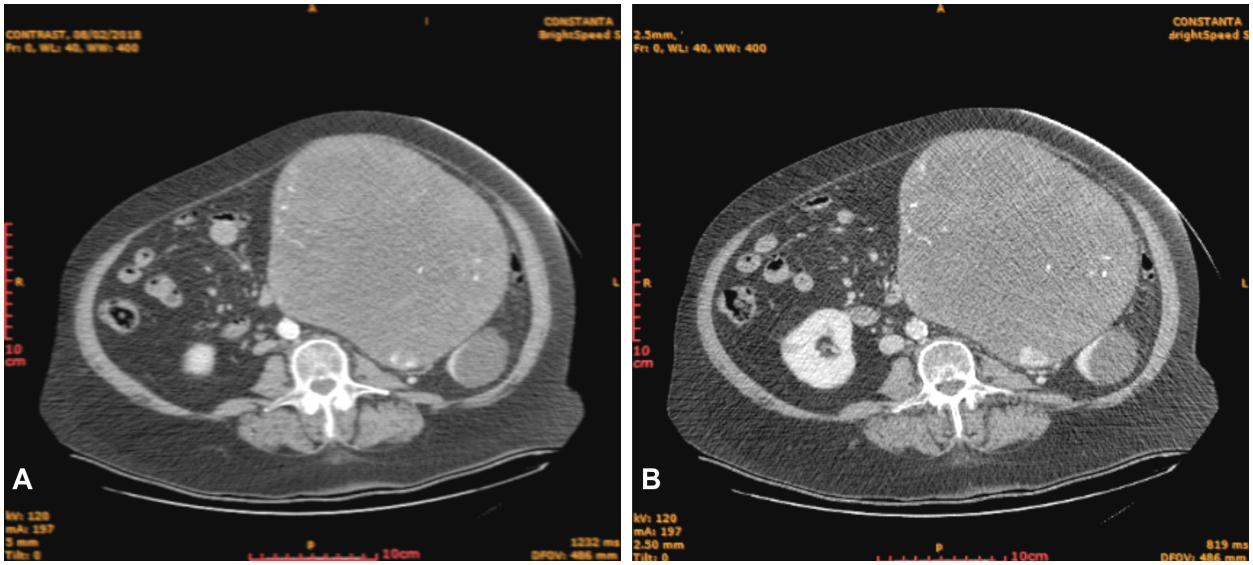

Figure 1 – ***(A and B) Native and contrast abdominal CT: macronodular retroperitoneal mass, inhomogeneous, with the presence of infracentrimetric calcifications. CT: Computed tomography.***

Laboratory findings included: mild hyperglycemia, mild thrombopenia, and slight elevation of aspartate aminotransferase (ASAT), of gamma-glutamyl transpeptidase (GGT) and of C-reactive protein (CRP). As ovarian tumor was supposed, carbohydrate antigen 125 (CA 125) and carcinoembryonic antigen (CEA) were performed. Only the level of CEA was slightly and clinically non-significantly elevated (Table 1).

Table 1 – ***Biochemical parameters***

Laboratory test	Value
White blood cells (nv: 4–10×10^3/μL)	7.8×10^3/μL
Hemoglobin (nv: 11.7–16.1 g/dL)	12.4 g/dL
Platelet count (nv: 150–450×10^3/μL)	137×10^3/μL
ALAT (nv: <33 IU/L)	28 IU/L
ASAT (nv: <32 IU/L)	40 IU/L
GGT (nv: <40 IU/L)	91 IU/L
CRP (nv: <0.5 mg/dL)	1.46 mg/dL
Glycemia (nv: 60–99 mg/dL)	118 mg/dL
CEA (nv: <5 ng/mL)	6 ng/mL
CA 125 (nv: <35 IU/mL)	23.3 IU/mL

ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase; CRP: C-reactive protein; CEA: Carcinoembryonic antigen; CA 125: Carbohydrate antigen 125; nv: Normal value.

The patient was transferred to the IInd Surgical Department of the same Institution and surgery was performed. A large, 330 mm, encapsulated tumor, was found (Figure 2). The tumor's origin was not clearly established during surgery.

The macroscopic description of the tumor, in the Department of Pathology was: encapsulated, grey-brown with red and yellow areas, with partial detachment of the capsule. On section, there were solid areas reported, alternating with cystic areas filled with blood. At the periphery, beneath the capsule, there were some yellow thin zones reported.

Microscopically, under the fibrous capsule, the pathologists described round to oval clusters and short trabeculae of relative small cells with pale eosinophilic cytoplasm and dark nuclei, constituting zona glomerulosa of the adrenal cortex.

Beneath these cells, the adrenal cortex was replaced by a proliferation of large, irregular, interconnected vessels, delineated by a single layer of endothelial cells. There were large areas of hemorrhagic necrosis, congestion, thrombosis and cystic degeneration, reported. Between the vessels, there were variable thickened fibrous septa, some with hyalinization and focal dystrophic calcifications (Figure 3).

Immunohistochemistry (IHC) [Ventana BenchMark Gx System; ready-to-use antibodies: cluster of differentiation 34 (CD34)/clone QBEnd 10 – Ventana; calretinin/clone SP65 – Ventana; melan A/clone A103 – Ventana) revealed positive CD34 reaction for endothelial cells within vascular proliferation (Figure 4) and positive adrenal cells for calretinin (Figure 5) and melan A (Figure 6).

Patient's recovery after surgery was uneventful and she was discharged 14 days after. Abdominal US performed after six months did not reveal any pathological modifications besides the previously existing, CT revealed renal cysts (Figure 7).

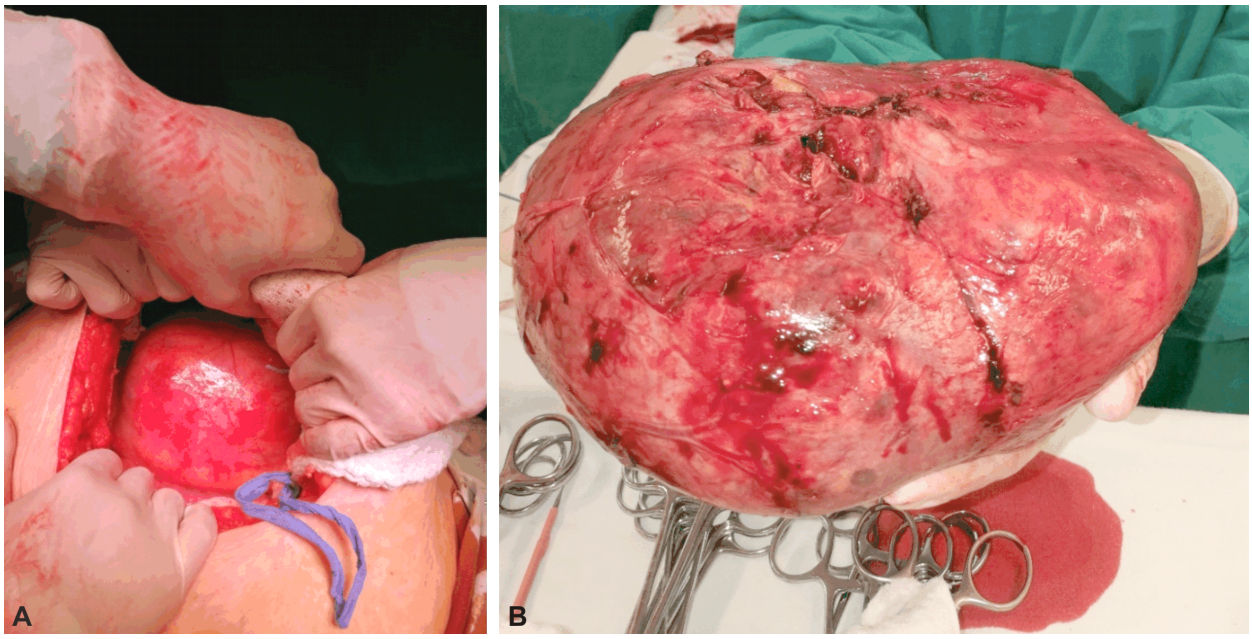

Figure 2 – *(A and B) Intra-operative macroscopic aspect: encapsulated tumor of 330 mm, with red and yellow areas, with partial detachment of the capsule.*

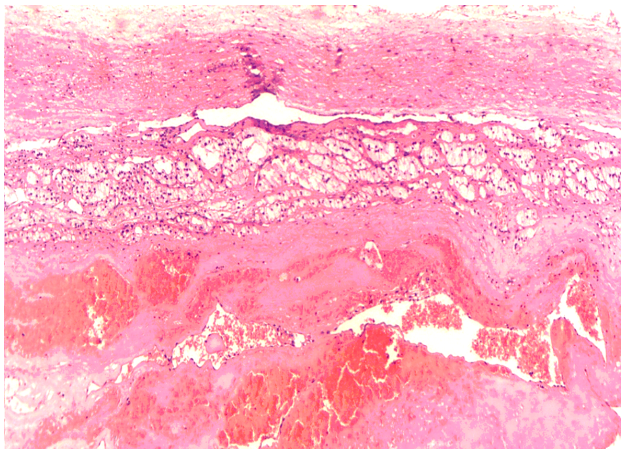

Figure 3 – *Adrenal cells beneath the capsule, in zona glomerulosa of the adrenal cortex and subjacent large vascular proliferation, with thrombosis and hemorrhagic necrosis (HE staining, ×100).*

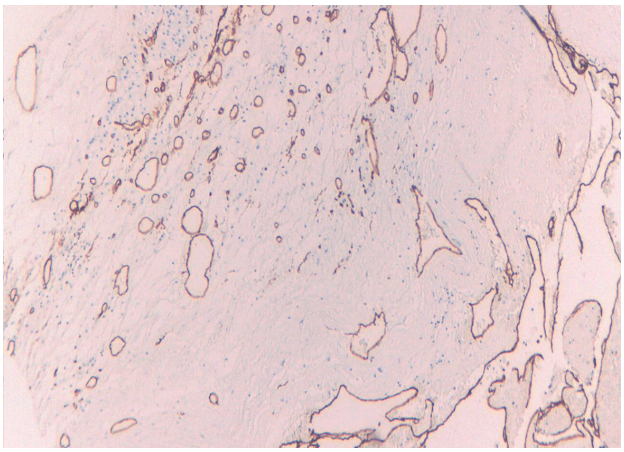

Figure 4 – *CD34: intense positive membrane reaction in endothelial cells; negative in adrenal cells (Anti-CD34 antibody immunomarking, ×10). CD34: Cluster of differentiation 34.*

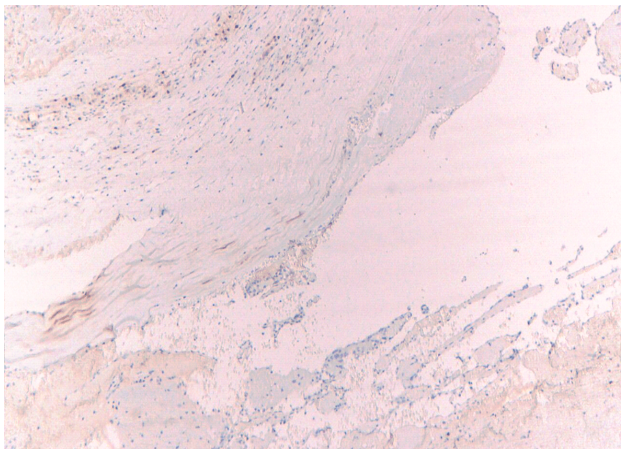

Figure 5 – *Calretinin: variable positive nuclear and cytoplasmic reaction in adrenal cells; negative in vascular proliferation (Anti-calretinin antibody immunomarking, ×10).*

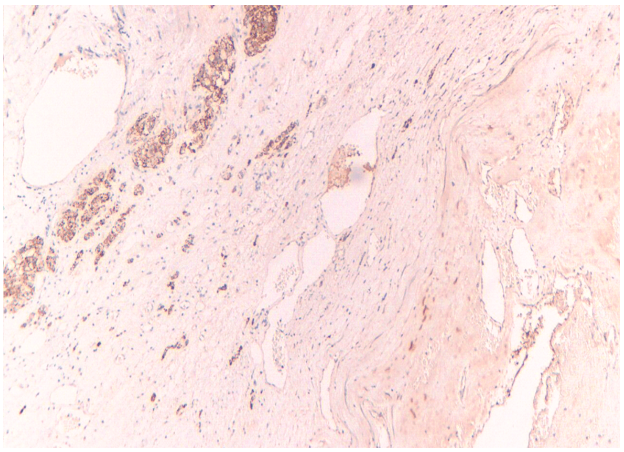

Figure 6 – *Melan A: positive reaction in the cytoplasm of the adrenal cells; negative in vascular proliferation (Anti-melan A antibody immunomarking, ×10).*

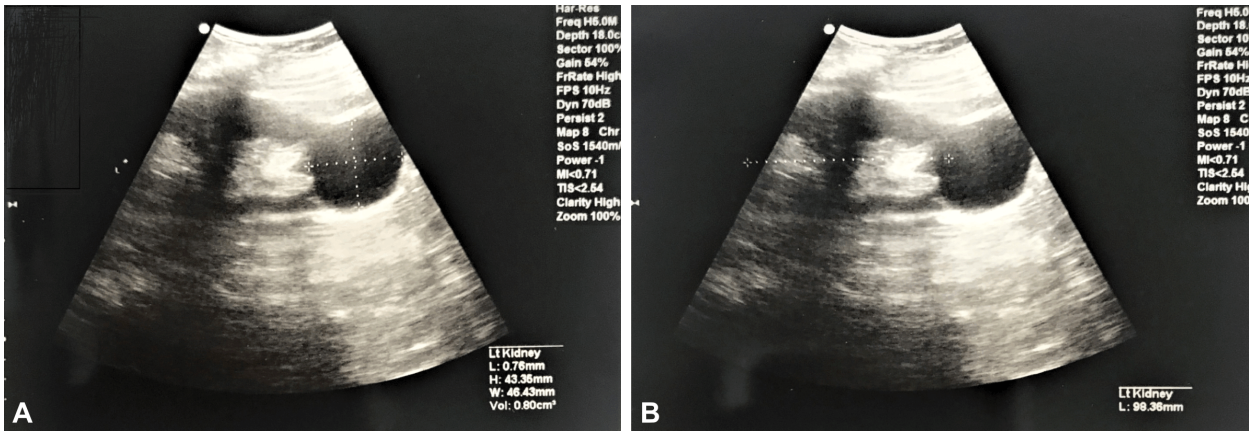

Figure 7 – ***(A and B) Ultrasound image of the left kidney revealing a normal aspect, with the exception of a homogenous transechoic, ovoid lesion of 46.43/43.35 mm, with posterior enhancement, at the superior renal pole, representing a previous existing cyst.***

Discussions

The adrenal cavernoma represents a rare non-malignant tumor, involving twice the feminine gender, usually between 50 and 70 years old. This tumor is symptomless most of the time, and is mainly diagnosed as an incidentaloma during an abdominal CT scan performed for another pathology. When cavernoma grows to giant dimensions, it can induce pain in the hypochondrium or flank, sensation of precocious satiety, impaired bowel movement due to compression and increasing abdominal pressure. Only three cases in the medical literature were described to be functional, one with glucocorticoid secretion and two with mineralocorticoid secretion [10, 11].

Discovered usually by US imaging, as an unspecific cystic tumor, generally with calcifications, more specific imaging techniques, as magnetic resonance imaging (MRI) and CT scan, are required in order to clarify the diagnosis.

MRI and contrast-enhanced CT usually reveal a peripheral spotty aspect, enhanced after contrast administration (due to the multiple peripheral cavities filled with blood), along with centripetal enhancement and with microcalcifications. The enhancement after contrast administration represents a typical feature of a hemangioma, not only in adrenals but also in other organs, too [12, 13]. Still, there are some cases in which this feature of enhancement is not present, making the diagnosis more difficult [12]. Microcalcifications are due to phleboliths' presence.

In our case, the difficulty consisted both in removing and in specifying the origin of cavernoma, as the big dimensions of the tumor made it difficult both for the surgeon and for the pathologist. The result of the CT examination did not offer enough information in order to clarify the etiology of the tumoral mass and reported smaller sizes than the ones discovered during the surgery.

Several carcinomas (breast, lung, gastrointestinal, renal, skin) can induce metastasis in the adrenal glands. Therefore, the knowledge of the tumoral adrenal mass's etiology is very important in the preoperative staging, if the patient is known with malignancy. Only four cases of independent adrenal hemangiomas coexisting with cancers of other organs (non-small-cell lung cancer, common bile duct cancer, breast cancer and gynecological cancer) are reported in the literature [14]. Microcalcifications revealed by CT scanning cannot differentiate between the histological types of adrenal tumors: carcinoma, tuberculosis, metastases from melanoma, hemorrhagic degeneration inside the tumor, etc. [14].

Typical immunohistochemical inspection of adrenal cavernoma is positive for CD31, CD34, and for blood coagulation factor VIII, indicating their endothelial nature [15]. In our case, the IHC was positive for CD34, and in correlation with the microscopic histopathological examination differential diagnosis with endometriosic cysts [16, 17], splenic hemangiomas, anastomosing hemangioma of the ovary [18], malignant ovarian tumors [19], multilocular cystic renal neoplasms [20], were taken into consideration. It was the positivity for calretinin and melan A that finally confirmed the adrenal origin of the cavernoma.

The treatment of adrenal incidentalomas depends on the size of the tumor, the suspicion of malignancy (based on imagistic aspect) or the functional status. In 2016, the *European Society of Endocrinology*, in collaboration with the *European Network for the Study of Adrenal Tumors* (ENSAT) published clinical practice guidelines for adrenal incidentaloma management, in which it is stipulated that open adrenalectomy must be performed for unilateral adrenal masses with radiological findings suspicious of malignancy and signs of local invasion [21].

In our case, due to the large volume of the tumoral mass, nor US, neither contrast enhanced CT scan were able to detect the adrenal origin of the tumor. Therefore, we started with the suspicion of a giant ovarian cyst. Because of this suspicion, no adrenal hormonal assessment was performed but the patient did not present the clinical aspect of any adrenal dysfunction, anyway. The final diagnosis came from the histopathological and immunohistochemical examination, the prognosis of patient's treatment relying over that.

Conclusions

The presented case supports the first conclusion – that, even if rare, hemangioma of the adrenal gland must be considered during the differential diagnosis of an adrenal tumor. We consider this case presentation important,

first, due to its rarity (less than 100 cases reported, only one with comparable sizes). Second, this article may assist clinicians, in dealing with the problems of differential diagnosis regarding the possible malignancy, as well as in choosing the surgical solutions. Last, we stress the importance of the collaboration between clinicians and pathologists.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

Costin Niculescu & Zizi Niculescu equally contributed to the manuscript.

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Case Report

Histiocytosis X and Pericarditis - A Rare Association and a Difficult Diagnosis

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ABSTRACT: Histiocytic disorders are a group of rare diseases with systemic involvement and with multiple clinical manifestations. We present the case of a 51 years old patient investigated for dyspnea with orthopnea, dry cough, asthenia, muscular weakness and ataxia. The association of previous symptoms with skin lesions, diabetes insipidus, partial hypophyseal insufficiency and pericarditis induced many diagnostic debates. The diagnosis is Histiocytosis X must be sustained by tissue biopsy with immunohistochemical assay or genetic testing. The particularity of our patient is the presence of pericarditis, rarely associated with histiocytosis. Collaboration between medical specialties is mandatory in order to treat this disorder.

KEYWORDS: *pericarditis, histiocytosis X, xantelasma, diabetes insipidus, pituitary failure*

Introduction

Histiocytic disorders are a group of rare diseases (1-2 adults per million people) that occur when there is an accumulation of monocytes, macrophages, and dendritic cells that can lead to organ damage and tumor formation.

Over the last 5 decades, because of the many clinical manifestations and of the variable severity depending on organ involvement, these disorders carried different names. [1]. For instance, Hand-Schüller-Christian disease, the eosinophilic granuloma and Abt-Letterer-Siwe disease were considered different entities, but now, it has been proved that they are the manifestations of a single entity: Langerhans cell histiocytosis (LCH).

The overall male-to-female ratio is 1.5:1. In case of involvement of only one organ, the male-to-female ratio is 1.3:1, while in case of multisystem disease the male-to-female ratio is 1.9:1. [2]. LCH can occur in individuals of any age. [3, 4, 5, 6, 7, 8, 9, 20]

The classification of histiocytic disorders the World Health Organization (WHO) [11] has proposed it as follows:

- Class I - Langerhans cell histiocytosis - most common
- Class II
  - Histiocytosis of mononuclear phagocytes other than Langerhans cells
  - Familial and reactive hemophagocytic lymphohistiocytosis (HLH)
  - Sinus histiocytosis with massive lymphadenopathy (SHML), Rosai-Dorfman disease
  - Juvenile xanthogranuloma (JXG)
  - Reticulohistiocytoma
- Class III
  - Malignant histiocytic disorders
  - Acute monocytic leukemia (FAB M5)
  - Malignant histiocytosis
  - True histiocytic lymphoma

Clinical presentation varies significantly. Lungs are affected in 20% up to 40% of patients with nodular infiltrate, pleural effusion, cystic changes, all evolving towards pulmonary fibrosis. Main symptoms are: dyspnea, cough, tachypnea [12]. Brain can be infiltrated, including cerebellum, with ataxia and loss of coordination, disorders of the hypothalamic and pituitary functions manifested as diabetes insipidus and total or partial hypopituitarism [13, 14, 15]. Cutanous manifestations are found in up to 50% of patients with LCH and they may present as maculoerythematous skin infiltrates, nodular popular, petechial xantomatous. Bones can be affected: singular or multiple osteolytic lesions of vertebrae, orbits, skull, long bones, sometimes with important periosteal reaction. Lymph nodes enlargement and gastrointestinal bleeding or liver dysfunction can also occur but in lesser extent.

The etiology of these disorders is not entirely known. It seems that many factors are involved: genetic, triggered by an infection (most frequently viral), cellular or immune

dysfunction, neoplastic mechanisms etc. [16, 17, 18, 19].

The diagnosis is based on clinical assumptions but it must be confirmed either through skin biopsy, bone marrow biopsy, lung biopsy [revealing LCH cells positively stained with antibodies to CD1a, S100 and/or anti-langerin (CD207)] or by genetic proof of BRAF mutations [20].

Even if sometimes, quite rare, the disease can be self-limited, in the majority of cases, especially with multiple organs involvement, it cause significant mortality. The main treatment in LCH is chemotherapy (usually Vinblastine) associated with corticoid treatment [21].

The prognosis depends on the severity of symptoms and on the multitude of involved organs. Usually, a good prognosis is associated with a good response to treatment in the first six weeks.

Case presentation and discussions

We present the case of patient of 51 years old, female, who was admitted in the IInd Medical Clinic of the Clinical Emergency Hospital of Constanta in October 2016 for dyspnea at rest with orthopnea, dry cough, important asthenia, muscular weakness and ataxia.

From her medical history we retain that 11 months before the actual admission the patient suffered two episodes of pericarditis and pleurisy which required pericardiocentesis and, in the same time, she was diagnosed, also 11 months ago, with pulmonary fibrosis, pituitary adenoma stage I/II Hardy, hyperprolactinemia, central diabetes insipidus and partial hypophyseal insufficiency. Treatment with desmopresine and cabergoline was initiated at the time.

Physical examination revealed an overweight patient with xantelasma and xantoma, with multiple macular areas and pigmented nevi with dimensions between 2 and 9 mm, spread on the entire body. The patient had important ataxia and mild cognitive disorder. Respiratory system's examination revealed bilateral subcrepitant rales and abolished breath sound in the left pulmonary base. Heart sounds were weak and tachycardic and mild jugular veins distension observed. No galactorrhea was found, either the moment of examination, or in her history, as well as no visual field disorders but a goiter of Ib degree with multinodular appearance. Patient's fluid ingestion was controlled under treatment with 0.1 mg of desmopresine.

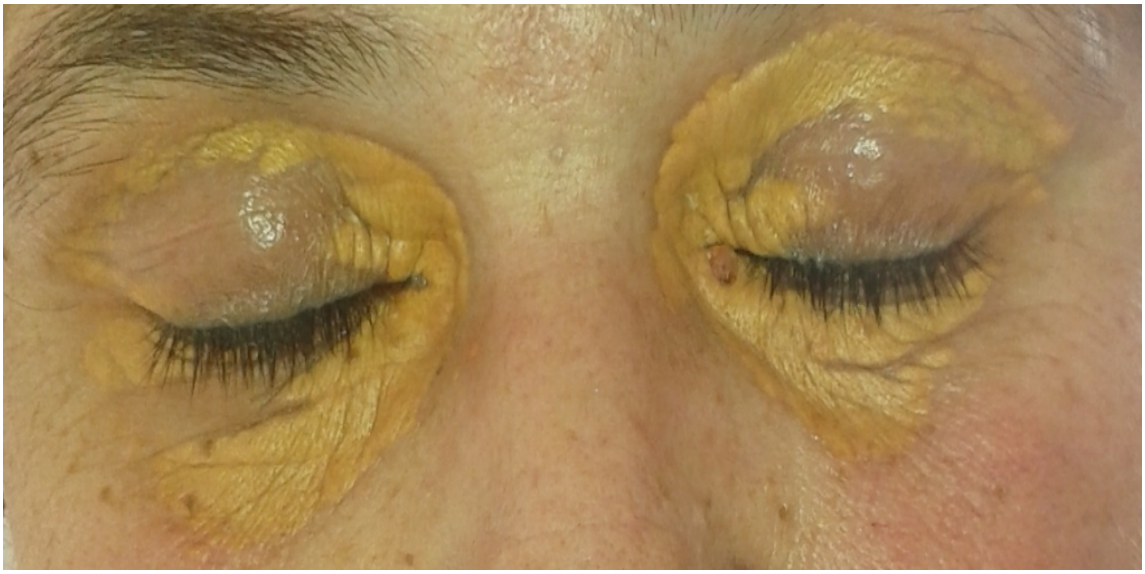

Fig.1. Xantelasma

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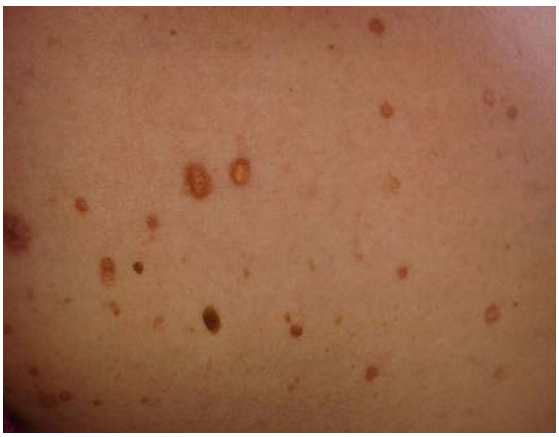

Fig.2. Generalised skin eruption (dermatofibromas)

Chest X-Ray and thoracic computed tomography (CT) confirmed the presence of pericarditis in medium amount, left pleural effusion, pulmonary fibrosis.

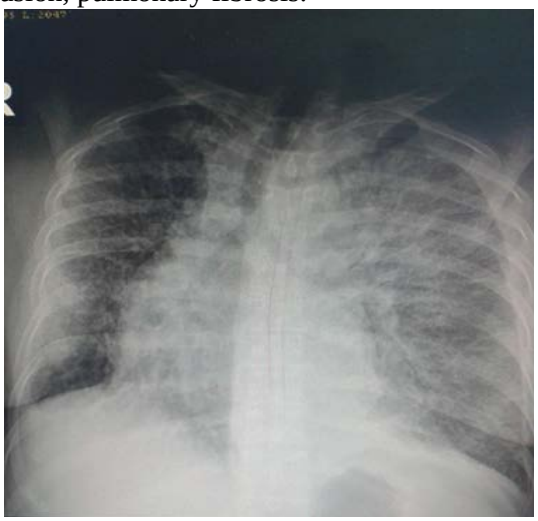

Fig.3. Chest X-Ray revealing enlarged heart and pulmonary fibrosis

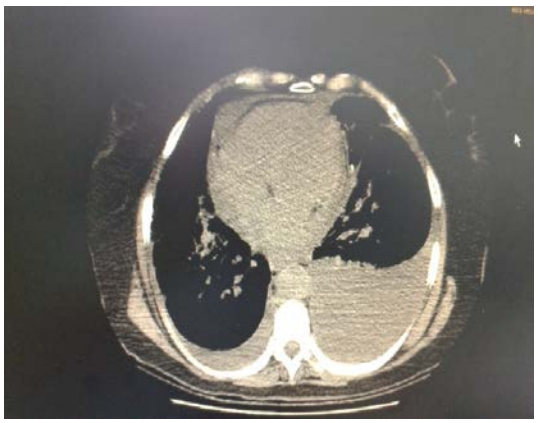

Fig.4. Thoracic CT revealing pericarditis and left pleurisy

Laboratory investigations revealed elevated ESR, serum fibrinogen and C reactive protein, leukocytosis with neutrophilia, mixed dyslipidemia, negative HIV tests, low PRL, FSH, LH, IGF-1, 25-OH-D2 vitamin, normal values of serum cortisol, TSH, FT4, ACTH, PTH, angiotensin convertase, serum calcium.

Table 1. Umoral parameters of the patient

Hb (g/dl)	13.3	11.7-16
White blood count (elements/mm^3)	22550	4000-10000
Platelets (elements/mm^3)	717000	150000-450000
Hematocrite (%)	41.5	35-47
ESR (mm/h)	35	<20
Fibrinogen (mg/dl)	574	200-400
CRP (mg/dl)	6.99	<0.5.
Glycemia (mg/dl)	99	<100
ALAT (ui/dl)	4	<33
ASAT (ui/dl)	20	<35
Urea (mg/dl)	32	<43
Creatinine (mg/dl)	0.63	<1
Cholesterol (mg/dl)	206	<200
Tryglicerides (mg/dl)	227	<150
Serum proteins (g/dl)	6.6	6.6-8.7
PRL (µUI/ml)	9	127-637 (under cabergoline treatment)
TSH (µUi/ml)	1.39	0.27-4.2
FT4 (pmol/l)	11.9	10.6-22.7
Serum cortisol am (nmol/l)	357	171-536 (7-10 am)
FSH (mUI/ml)	3.1	25.8-134.8 (menopausal)
LH (mUI/ml)	0.8	7.7-58.5 9menopausal)
Serum Na (mmol/l)	141	136-145
Serum K (mmol/l)	4.9	3.3-5.1
PTH (pg/ml)	25.2	15-65
Phosphoremia (mg/dl)	4.1	2.5-4.5
ACTHpg/ml	9.35	7.2-63.3
IGF -1 (ng/ml)	51.23	57-236
25-OH vitamin D (ng/ml)	10.4	30-100
p ANCA (u/ml)	<0.7	<7
c ANCA (u/ml)	<0.2	<7
C3c (mg/dl)	132	90-180
C4 (mg/dl)	28	10-40
ACE (u/l)	15	12-68
Ca ionic (mg/dl)	4.3	3.82-4.82
Serum total Ca (mg/dl)	8.6	8.6-10
Antibodies anti HIV 1+2	negativ	

ACE=angiotensin-converting enzyme
ACTH=adrenocorticotropic hormone
ALAT=alaninaminotransferaze
ASAT=aspartataminotransferaze
CRP=C reactive protein
cANCA=antibodies anti-proteinase 3
pANCA=antibodies anti myeloperoxidase
FSH=follicle stimulating hormone
LH=luteinizing hormone
PRL=prolactine
PTH=parathormone

Thyroid ultrasound confirmed the micronodular aspect of the thyroid.

The cardiologic examination confirmed the presence of moderate pericarditis with indication of medical treatment, and, taking into consideration the two previous episodes of pericarditis with pericardiocentesis, a suspicion of a systemic disease as a possible etiology was issued.

The neurologic examination confirmed ataxia and the cerebellar syndrome. From neurologic point of view, the skin eruption corroborated

with the neurologic syndrome induced the suspicion of neurofibromatosis. Related to this suspicion, ophthalmologic, dermatologic and cerebral MRI were performed.

The cerebral MRI revealed cerebral, cerebellar, thalamic, and tentorial intranevraxial lesions with possible granulomatous etiology, as well as a nodular lesion of the upper part of the pituitary stalk and of the hypothalamic infundibulum, with impression on the optical chiasm.

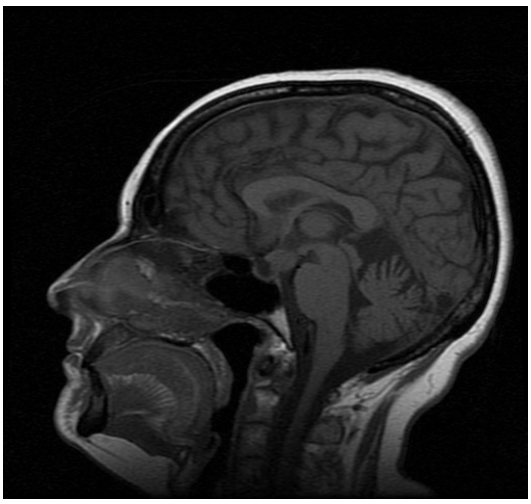

Fig.5. Hypothalamic-hypophyseal MRI revealing nodular lesion of the upper part of the pituitary stalk and of the hypothalamic infundibulum, with impression on the optical chiasm T1

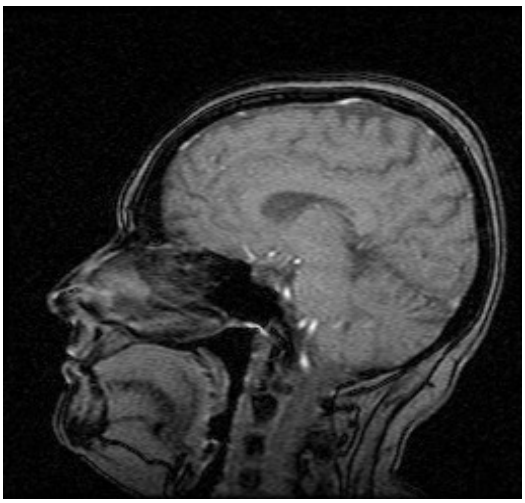

Fig.6. Hypothalamo-hypophyseal MRI revealing nodular lesion of the upper part of the pituitary stalk and of the hypothalamic infundibulum, with impression on the optical chiasm T2

Rheumatologic examination performed in the context of a possible systemic disorder and of the cerebral MRI aspect as well as on the presence of an important inflammatory syndrome, failed to confirm the suspicion of SLE or Wegener granulomatosis based on a normal panel of antibodies (Anti DNA ds antibodies, ANA, pANCA, cANCA, C3, C4), but required skin biopsy.

The suspicion of dermatofibroma was raised by dermatological examination, instead of neurofibromatosis. Skin biopsy was performed and the histopathological result confirmed dermatofibroma (benign fibrous histiocytoma).

Based on the association of skin lesions, lung disorders and on the involvement of hypothalamic-pituitary region and neurodegenerative changes in the cortex, cerebellum and tentorium, on the presence of diabetes mellitus, we took into account another suspicion of diagnosis in this patient: Histiocytosis X. Unfortunately we couldn't sustain this diagnosis because of the absence of Langerhans cells in the histopathological examination, because of the impossibility of performing cell markers and phenotypes of histiocytic and related disorder and because the patient refused bronchoscopy, bronchoalveolar lavage and bone marrow biopsy which might have provided the histopathological diagnosis.

The patient was treated with antibiotics due to the respiratory symptoms correlated with leukocytosis and inflammatory syndrome, cabergoline 0.25 mg twice a week, desmopresin, 0.1 mg/day, fenofibrate 160 mg/day, Dexametazone 8 mg per day for 5 days. Medrol in dosage of 4 mg per day was tried for 2 weeks but, because of no improvement in patient's neurologic or respiratory status, corticoid treatment was tapered to zero.

Patient's evolution under the above mentioned treatment is not satisfactory, even if from endocrinological point of view the patient is compensated, the respiratory function worsens.

We still take in consideration the suspicion of Histiocitosis X but further investigations are necessary: bone marrow biopsy to reveal LCH cells positively stained with antibodies to CD1a, S100 and/or anti-langerin (CD207) or detection of BRAF V 600 mutations. [22]. Several attempts were made to involve the oncologic department in the investigations and treatment procedures, but without the possibility of performing the above mentioned investigations, the patient was not included in chemotherapeutic treatment.

A particularity of our patient is the presence of pericarditis. The main symptoms of our patient (dyspnea with orthopnea, dry cough) are related to the association between pericarditis and pulmonary fibrosis. We found only three other cases of pericarditis in LCH patients in

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medical literature: 2 children and one adult. [22, 23, 24]

Conclusions

Histiocytosis X or Langerhans cell histiocytosis is a rare disease with a difficult diagnosis.

Even if the suspicion is based on clinical manifestations, tissular biopsy with immunohistochemical assays or genetic evaluation are required in order to consider the diagnosis.

The association between pericarditis and histiocytosis x is rare and challenges the treatment options.

Treatment requires strong interdisciplinary collaboration between endocrinologist, oncologist, hematologist, pneumologist, orthopedic surgeon and, sometimes, cardiologist, as is in our case.

Acknowledgements

All authors had equal contribution.

Conflict of interests

The authors declare that they have no conflict of interests.

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