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REVIEW

The Early Repolarization ECG Pattern – An Update

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Early repolarization pattern (ERP), a form of J-wave syndromes, was considered long time a benign ECG phenomenon. However, recent data confirmed that certain phenotypes of ERP are related to an increased risk of sudden cardiac death (idiopathic ventricular fibrillation). Our paper gives a short and practical update regarding the main issues related to ERP: epidemiological data, molecular and electrophysiological background, clinical significance and risk stratification. At the end, the future directions of research and clinical management related to ERP are presented.

Keywords: J-wave, early repolarization syndrome, sudden cardiac death

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Introduction. History

From the beginning of electrocardiography, so called idiopathic J-waves were found on the ECG recordings of many species, including humans. The J-point on ECG is defined as the transition between the end of the QRS complex and the ST segment. Deviation of this point from the isoelectric line determine the presence of J-deflection (wave), which could be followed by upsloping or horizontal ST-segment elevation, determining the appearance of early repolarization ECG pattern (ERP). The ERP was first described in 1936 by Shipley and Hallaran, who studied 200 young healthy men and women and described the J-deflection as notching of the descending part of the QRS complex [1-3].

ERP was considered previously as a benign, "innocent", ECG phenomenon, as a distinct form of the so called J-wave syndromes (which include also the Brugada syndrome). However, in the last ten years, epidemiological and case-control studies found some forms of ERP to be predictors and risk factors for sudden cardiac death (primary ventricular fibrillation). This is particularly true, when the ST segment has a horizontal or descending direction in the inferior and/or infero-lateral leads. The risk is even higher when the amplitude of J-wave is greater than 0.2 mV [2].

Diagnostic criteria. Prevalence

Several consensus papers were issued on diagnostic criteria of ERP. The last criteria are summarized in Table I [4].

Early repolarization syndrome is a term used for those patients who present ERP on the 12-lead ECG and had a previous episode of aborted sudden cardiac death or had documented malignant ventricular arrhythmias (ventricular fibrillation, polymorphic ventricular tachycardia) [2].

The prevalence of ERP in the general population is between 1% and 9%, but is 15% to 70% in patients with idiopathic ventricular fibrillation. Observing different patient cohorts, ERP produced an increase of 4 to 10 fold in the occurrence of sudden cardiac death, the maximal incidence being reached between 35 and 45 years [5, 6].

Regarding the influence of gender, ERP is strongly associated with male sex, men representing more than 75% of cases. Also, in male subjects a greater J-point elevation was observed than in women, and males represent 75% of the malignant cases. Surawicz et al. set a hormonal hypothesis after evaluating the ECGs of 529 males aged 5 to 96 years. They observed that the prevalence of ERP increased parallelly with the rise of testosterone levels during puberty. In elderly males, when testosterone levels are in decline, the prevalence of ERP is decreased [5, 7].

ERP is more common in young and physically active individuals, a phenomenon which regress with age. In black people this pattern is particularly prevalent, however, these subjects were underrepresented in diverse studies and their arrhythmic risk is still undetermined. In athletes ERP shows a higher prevalence than in the general population: a presence in 20% of noncompetitive and almost 90% in competitive athletes [5, 6].

Table I. Current diagnostic criteria of ERP [4]

1	Presence of J-wave - notching at the end of the QRS complex (Fig. 1) or slur on the positive R-wave descending limb (Fig. 2), with or without ST-segment elevation
2	J-wave peak amplitude greater than 0.1mV, present in ≥2 contiguous leads, except the V1-V3
3	QRS duration less than 120 ms in the leads not containing notching or slur

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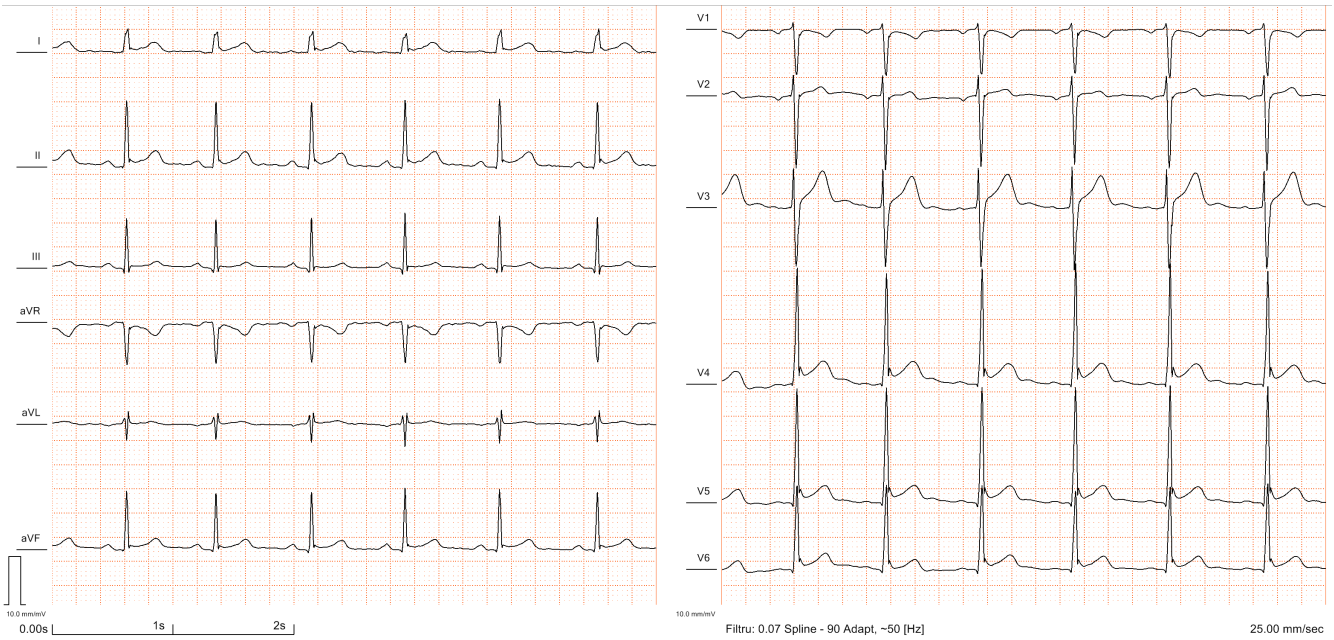

Fig. 1. ECG registration with J-waves appearing as notching at the end of the QRS complex, best visible in V4-6 leads (from the personal collection of Dr. Frigy A.)

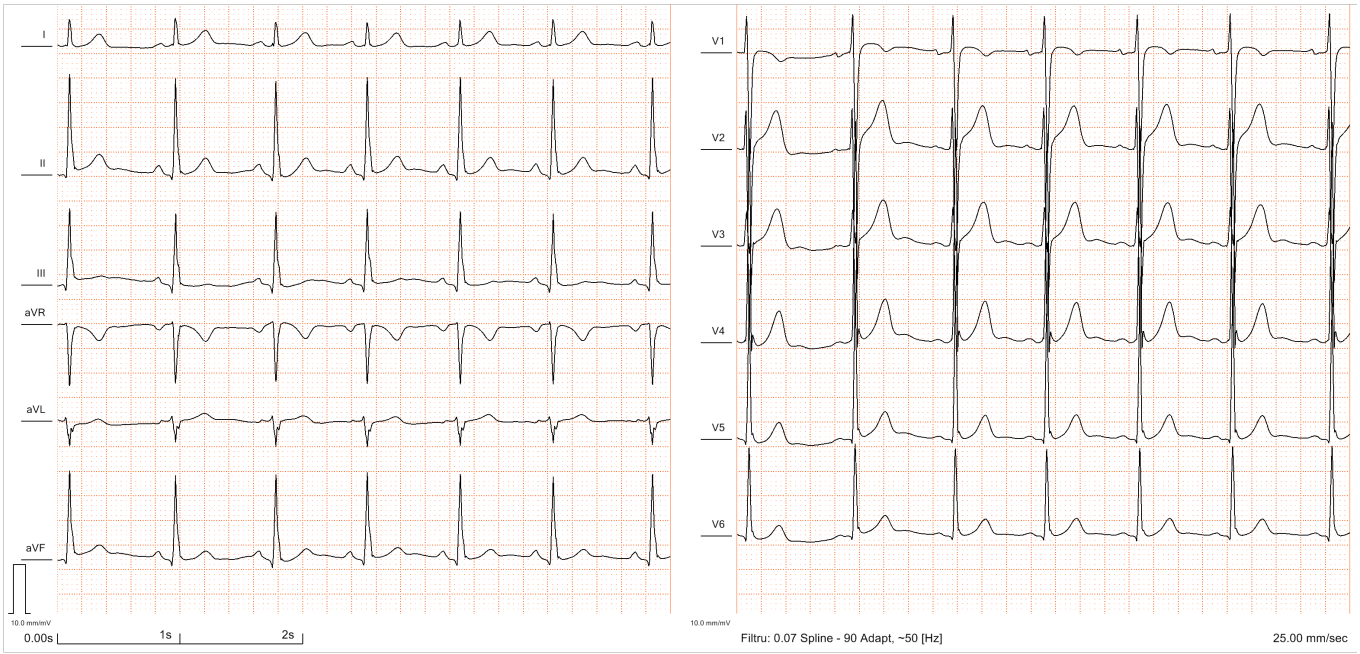

Fig. 2. ECG registration with J-waves appearing as slur on the positive R-wave descending limb in infero-lateral (II, III, aVF, V5,6) leads (from the personal collection of Dr. Szabó I.A.)

Molecular basis. Electrophysiological background

Antzelevitch et al. in the 1980s performed an experiment in canine, founding a heterogeneous transmural distribution of the transient outward potassium current (I_{to}) in the ventricular myocardium. Their hypothesis was the following: for the appearance and shape of the J-wave the transient outward potassium current is responsible, which, being not uniformly active in the myocardium, produces differences between the epicardial and endocardial action potential (AP) shapes [3, 8].

The normal epicardial AP of the ventricular myocardium differs from that of endocardial, having a prominent phase 1 notch or a typical spike-and-dome morphology. This is due to the fact that during the 1st phase of AP there is a large I_{to} in the epicardium, producing a more intense initial repolarization. In the case of early repolarization there is an increase in the difference in AP amplitude at the endocardial-epicardial level, which manifests as J-wave (Fig. 3). This wave corresponds to the current flowing from the endocardium to epicardium during phase 1. The characteristic notch on the epicardial action potential coincides with the J-wave on the surface ECG, also, they

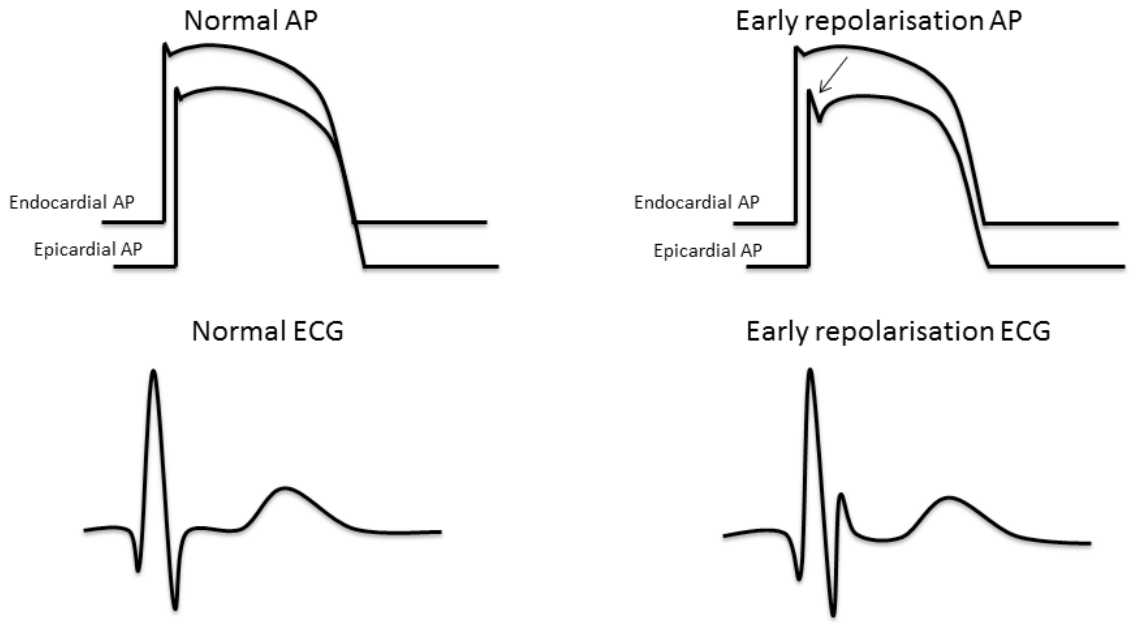

Fig. 3. Electrophysiological basis of the appearance of J-wave on surface ECG (see explanations in text) [9]

change parallelly, ventricular activation clearly influencing the appearance of J-wave [8, 9].

The phenomenon could be more enhanced in the setting of mutations of genes encoding inward Na^+, Ca^{2+} or outward K^+ currents. The functional properties of these mutations have not been determined definitively. Independently of the initiating mechanism, the ventricular tachyarrhythmia is maintained by local transmural reentry induced by the increased AP gradients. The genetic basis for early repolarization syndrome is not entirely clarified, but today's data show seven different genes encoding cardiac ion channels that are associated with early repolarization syndrome, more exactly, 3 K^+ channel related, 3 Ca^{2+} channel related and 1 Na^+ channel related genes were found to be involved [2, 9].

The first reported gene was KCNJ8 which encodes the inward rectifying K^+ channel Kir6.1, one of the subunits of the cardiac ATP-sensitive K+ channel. A gain-of-function mutation of KCNJ8 - S422L - was first identified in a 14-year-old Caucasian female who suffered of recurrent ventricular fibrillation [10].

Clinical significance. Risk stratification

ERP, known as notch or slur of the downsloping limb of the QRS complex, was considered long time a benign and innocent ECG phenomenon. The benign nature of ERP was challenged in 2000, based on experimental data showing that the presence of this ECG manifestation predisposes to the development of polymorphic ventricular tachycardia and ventricular fibrillation [11]. Validation of this hypothesis was provided 8 years later by Haissaguerre et al., Nam et al., and Rosso et al. These studies, together with other case-control and population-based studies demonstrated the increased risk for development of malignant arrhythmias and sudden cardiac death in patients having ERP, especially localized in the inferior and infero-lateral leads. However, the appearance of J-waves on a routine, screening ECG does not have automatically the significance of "high risk" for malignant arrhythmic events, because the odds for ventricular fibrillation are as low as 1:10,000. Rosso et al. stated that the occurrence of a J wave on the surface ECG increases the probability of ventricular fibrillation from 3.4:100,000 to 11:100,000. [11-15] It is important to mention, that there are emerging data concerning the ventricular arrhythmia promoting effect of ERP in patients with chronic coronary heart disease and in the setting acute myocardial infarction [16, 17].

The most difficult task in managing ERP is risk stratification, the correct and feasible estimation of the risk of sudden cardiac death, to distinguish those features which

Table II Genetic background of ERP and early repolarization syndrome – the main genes involved and the corresponding ion channels [2]

	Gene	Protein	Ion channel	% of probands
K+ channel	KCNJ8	Kir6.1	IK-ATP	0.6
	ABCC9	SUR2A	IK-ATP	7.3
	KCND2	Kv4.2	Ito	2.0
Ca 2+ channel	CACNA1C	Cav1.2	Ica	4.1
	CACNB2b	Cavβ2b	Ica	8.3
	CACNA2D1	Cavα2d	Ica	4.1
Na+ channel	SCN5A	Nav1.5	INa	6.0

Table III. Schematic representation of the elements of risk stratification in patients with ERP. [2] Abbreviations: VF – ventricular fibrillation, VT – ventricular tachycardia, SCD – sudden cardiac death, VPBs – ventricular premature beats

	J-WAVE MANIFESTATIONS	
	Topography (ECG leads)	Clinical and other ECG features
Arrhythmic Risk	• Global presence • Right precordial (Brugada ECG pattern) • Inferior or infero-lateral • Lateral	• Resuscitation from cardiac arrest, documented VF, or polymorphic VT • Positive family history of SCD, arrhythmic syncope, gene mutations • Short-coupled VPBs • Fragmented QRS • Short QT • Dynamic changes in J-wave amplitude • J-waves >2mV • J-waves with horizontal /downsloping ST-segment • Tall R-waves, rapidly ascending ST segment

are related with an increased arrhythmic risk. Table III summarizes the most important elements - and their relative value - in risk stratification [2]. Tikkanen et al., in their papers stated that a rapidly ascending ST- segment after the J point in healthy athletes, seems to be a benign variant of ERP, while a horizontal or descending ST-segment elevation is associated with an increased risk of arrhythmic death [18, 19].

Present guidelines recommend (class I indication) an implanted cardioverter defibrillator (ICD) in survivors of an episode of ventricular fibrillation in the case of ERP (= early repolarization syndrome). But, we still miss firm recommendations for the treatment of patients with ERP who have only syncope of unknown origin, or are asymptomatic. The majority of patients with ERP does not require any intervention, but have to be carefully investigated. [2]

Primary prevention of sudden cardiac death in asymptomatic ERP is a major challenge. Present data suggest that a J-point elevation >0.2mV linking short QTc interval confers a 3-fold increase in sudden cardiac death risk. ICD implantation is recommended in patients with early repolarization syndrome who have survived a cardiac arrest, while quinidine may be helpful in those patients with ICD and frequent non-sustained ventricular tachyarrhythmias. In acute setting, another therapeutic approach is to increase heart rate beyond 90, up to 120 beats/min with isoproterenol infusion, which can suppress ventricular arrhythmias. In patients who are not responsive to pharmacological treatment endocardial ablation of ectopic foci in the early repolarization zone can be useful [2, 3].

Future perspectives. Conclusions

The main challenge for the future in the case of ERP consists of risk stratification. This is/has to be a complex task, based on multiple parameters: clinical, ECG and genetic features, biomarkers, etc. There are some promising new methods, especially for the identification of genetic (causative mutations) and electrical substrates of the malignant forms of ERP. ECGI (electrocardiographic imaging) is a translational research tool used in the setting of inherited arrhythmia syndromes. This technique records the body surface potentials using several electrodes and computes truly the electrical activity of epicardium. [8, 20, 21]

ERP is an ECG phenomenon which still remains controversal in many aspects: precise diagnostic and screening criteria, complex electrophysiological and genetic background, feasible risk stratification and prophylactic treatment. For the everyday clinician is very important to observe and identify the phenomenon and to make an initial risk stratification based on history and common ECG features.

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Conflict of interest

None to declare.

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RESEARCH ARTICLE

Risk Factors Associated with Acute Coronary Syndrome after Successful Percutaneous Coronary Intervention

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Objective: Admission for acute coronary syndrome after successful percutaneous coronary intervention is a delicate situation for the patient and doctor. Predictors of these cases are poorly described. **Methods**: We retrospectively analysed the files of post-percutaneous coronary intervention patients admitted to the Department of Cardiology of the Institute for Cardiovascular Disease and Heart Transplant in Tirgu Mures between January 2012 and December 2015. Analyses using the t-test, chi-square test, and Fisher test were performed to compare demographics, clinical and angiographic characteristics of patients with acute coronary syndrome, patients with stable angina, and those without symptoms. **Results**: One hundred eighty post-percutaneous coronary intervention patients were readmitted; 46 patients (25.55%) were readmitted for acute coronary syndrome. Histories of arterial hypertension and renal dysfunction at hospital admission were associated with acute coronary syndrome. Bare metal stent in-stent restenosis and localisation of bare metal stent in-stent restenosis of the left descendent coronary artery were angiographic predictors of acute coronary syndrome. **Conclusion**: Several clinical and angiographic factors identify patients at high risk for acute coronary syndrome after successful percutaneous coronary intervention. Recognition and treatment of these factors may prevent readmission for such a dangerous condition and may improve outcomes.

Keywords: acute coronary syndrome, percutaneous coronary intervention, in-stent restenosis

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Introduction

Coronary artery stents, both bare metal stents and drug-eluting stents, significantly reduce the incidence of events related to culprit lesion treated during the first (index) percutaneous coronary intervention; however, coronary artery disease is a continuous process. Reoccurrence of symptomatology, particularly acute coronary syndrome, is a challenging situation because acute coronary syndrome is still a major cause of death and has a high economic burden. Identification of factors that could predict the development of acute coronary syndrome, especially preventable factors, would be extremely useful for the clinical management of these patients.

Methods

This was a single-centre retrospective analysis of 180 readmitted post-percutaneous coronary intervention patients over a 4-year period (January 2012-December 2015). Patients were divided into three groups: acute coronary syndrome patients, patients with stable angina, and asymptomatic patients. Baseline characteristics, cardiac history, risk factors, comorbidities, results of coronarography at the index percutaneous coronary intervention and at readmission, stent type used at the index percutaneous coronary intervention, and medication after percutaneous coronary intervention were compared between the three subgroups.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation and compared using t-tests. Categorical variables were presented as numbers and percentages and compared using chi-square or Fisher tests. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using STATA 14.0 (Stata Corporation, College Station, TX, USA).

Results

Forty-six (25.55%) patients with acute coronary syndrome, 101 (56.11%) patients with stable angina, and 33 (18.33) asymptomatic patients were readmitted after successful percutaneous coronary intervention (Table I).

Patients with acute coronary syndrome were older than asymptomatic patients (63.93 ± 10.69 vs. 58.87 ± 9.25 years; p=0.031) and more often had a history of myocardial infarcts than patients with stable angina (p=0.005).

Arterial chronic hypertension and impaired renal function (estimated glomerular filtration rate ≤60 ml/min) were more frequent in the acute coronary syndrome group than in the other two groups. There were no other differences

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Table I. Baseline characteristics, cardiovascular risk factors, and comorbidities

	Stable angina group		Asymptomatic group		ACS group
	101 (56.11%)	*p	33 (18.33%)	*p	46 (25.55%)
Age, years	61.97 ± 9.72	0.273	58.87 ± 9.25	0.031	63.93 ± 10.69
Male, n (%)	76 (75.25)	0.652	27 (81.82)	0.301	33 (71.74)
HTN, n (%)	93 (92.8)	0.057	29 (87.88)	0.027	46 (100)
Diabetes mellitus, n (%)	19 (18.81)	0.066	6 (18.18)	0.152	15 (32.61)
Obesity, n (%)	28 (27.72)	0.946	10 (30.3)	0.844	13 (28.26)
Dyslipidemia, n (%)	50 (49.5)	0.586	12 (36.36)	0.114	25 (54.25)
Smoker, n (%)	14 (13.86)	0.578	4 (12.12)	0.752	8 (17.39)
eGFR ≤60 ml/min, n (%)	20 (19.8)	0.006	4 (12.12)	0.006	19 (41.3)
Previous MI, n (%)	43 (42.57)	0.005	18 (54.55)	0.246	31 (67.39)
Previous CABG, n (%)	6 (5.94)	1	0	0.261	3 (6.52)

*p compared with ACS group.

ACS, acute coronary syndrome; HTN, hypertension; MI, myocardial infarction; CABG, coronary artery bypass grafting; eGFR, estimated glomerular filtration rate.

in baseline characteristics, risk factors, and comorbidities between the three groups.

Right coronary artery disease was more frequent in the acute coronary syndrome group than in the other two groups; however, the numbers of diseased vessels between the three groups at the index procedure or readmission were not different. Drug-eluting stent utilisation at index percutaneous coronary intervention was more frequent in the asymptomatic group. Bare metal stent in-stent restenosis and localisation of in-stent restenosis to the left descending artery occurred more often in the acute coronary syndrome group than in the stable angina group or asymptomatic group (Table II).

Discussion

Arterial chronic hypertension is a classical and well-known cardiovascular risk factor for the development of atherosclerosis and coronary artery disease. A previous study revealed a 63.4% prevalence of hypertension among acute coronary syndrome patients [1]. The impact of hypertension on patients with acute coronary syndrome is related to the progression of coronary atherosclerosis and favours the

Table II. Intervention features

		Stable angina group		Asymptomatic group		ACS group
		N (%)	p*	N (%)	p*	N (%)
Number of diseased vessels at index PCI	1	50 (49.5)	0.301	10 (30.3)	0.279	18 (39.13)
	2	32 (31.68)		15 (45.45)		16 (34.78)
	3	16 (15.84)		6 (18.18)		12 (26.09)
	>3	3 (2.97)		2 (6.06)		0
Lesion localisation at index PCI	LMCA	3 (2.97)	0.377	0	0.261	3 (6.52)
	LAD	70 (69.31)	0.569	24 (72.37)	0.906	34 (73.91)
	RCA	40 (39.6)	0.056	19 (57.58)	0.926	26 (56.52)
	LCX	35 (34.65)	0.808	10 (30.3)	0.828	15 (32.61)
	Other coronary artery	24 (23.76)	0.385	11 (33.33)	0.102	8 (17.39)
Time interval between index PCI and readmission (mean ± median), months		27.68 ± 29.66	0.07	24.33 ± 35.12	0.09	37.86 ± 35.52
Number of diseased vessel at readmission	0	45 (44.55)	0.087	15 (45.45)	0.142	11 (23.91)
	1	37 (36.63)		14 (42.42)		21 (45.65)
	2	14 (13.86)		3 (9.09)		10 (21.74)
	3	5 (4.95)		1 (3.03)		4 (8.7)
Lesion localisation at readmission	LMCA	2 (1.98)	0.589	0	0.507	2 (4.35)
	LAD	22 (21.78)	0.161	6 (18.18)	0.152	15 (32.61)
	RCA	22 (21.78)	0.028	6 (18.18)	0.046	18 (39.13)
	LCX	7 (6.93)	0.226	5 (15.15)	1	6 (13.04)
	Other coronary artery	21 (20.79)	0.476	6 (18.18)	0.409	12 (26.09)
Stent type at index PCI	DES	34 (33.66)	0.081	16 (48.48)	0.006	9 (19.57)
	BMS	77 (76.24)	0.239	23 (69.7)	0.108	39 (84.78)
DES ISR, n (%)		4 (3.96)	0.258	0	0.136	4 (8.7)
BMS ISR, n (%)		34 (33.66)	0.033	6 (18.18)	0.002	24 (52.17)
BMS ISR localisation	LMCA	10 (9.9)	0.052	1 (3.03)	0.021	10 (21.47)
	RCA	8 (7.92)	1	2 (6.06)	1	3 (6.52)
	LCX	6 (5.94)	1	1 (3.03)	0.636	3 (6.52)
	Other coronary artery	3 (2.97)	0.648	1 (3.03)	1	2 (4.35)

*p compared with ACS group.

LMCA, left main coronary artery; LAD, left descendent artery; RCA, right coronary artery; LCX, left circumflex coronary artery; DES, drug-eluting stent; BMS, bare metal stent; ISR, in-stent restenosis.

development of vulnerable atherosclerotic plaques through which rupture can occur during acute coronary syndrome. The prognoses of patients with known hypertension and acute coronary syndrome are also impaired. In the Kamir registry, a history of hypertension was related to higher in-hospital mortality [2]. In the GISSI-2 study, in-hospital and 6-month mortality rates were higher for hypertensive patients than for normotensive myocardial infarction patients [3].

Chronic kidney disease is associated with accelerated atherosclerosis and is a predictor of cardiovascular morbidity, mortality, and all-cause mortality for patients with acute coronary syndrome [4,5]. Mechanisms related to the adverse outcomes are more severe vessel disease on presentation with acute coronary syndrome [4], differences in coronary plaque morphology [6], less aggressive revascularization, and medical therapy.

The superiority of drug-eluting stents compared to bare metal stents regarding target lesion revascularisation has been investigated in many studies. In NORSTENT, the largest randomised study to compare contemporary drug-eluting stents and bare metal stents, target lesion revascularisation and definite stent thrombosis were significantly lower for drug-eluting stent patients than for bare metal stent patients [7]. Furthermore, in our study, bare metal stent in-stent restenosis was more frequent than drug-eluting stent in-stent restenosis in the acute coronary syndrome group. These findings raise the question of the utility of bare metal stents in the era of drug-eluting stents and bioabsorbable vascular stents. High haemorrhagic risk and inadequate dual antiplatelet therapy may be reasons why bare metal stents are preferred to drug-eluting stents.

The localisation of bare metal stent in-stent restenosis was not unusual because tortuosity and angulation of the left descending coronary artery can predispose patients to accelerated progression of atherosclerosis [8.9].

Right artery disease was more frequent in the acute coronary syndrome group than in the other two groups at readmission. This raised the hypothesis of incomplete revascularisation at index percutaneous coronary intervention or progression of atherosclerosis after index percutaneous coronary intervention.

Study limitations

Our study had several limitations. First, the data were retrospectively extracted by reviewing medical observation files and depended on the accuracy and completeness of them. Second, the study included only patients admitted to our clinic. Patients admitted to other hospitals or who died were not included in our study. Finally, the time interval between index percutaneous coronary intervention and readmission has not been standardized. However, the average time interval for each group was not statistically significant.

Conclusion

Hypertension and impaired renal function are clinical risk factors for acute coronary syndrome. Right coronary artery disease, bare metal stent in-stent restenosis, and left descending artery localisation of bare metal stent in-stent restenosis are angiographic risks associated with acute coronary syndrome. Recognition and treatment, particularly of preventable factors, may improve the outcomes and prognoses of coronary disease patients after successful percutaneous coronary intervention.

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Conflict of interest

None to declare.

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RESEARCH ARTICLE

Lacosamide Reduces Seizure Severity but Increases Seizure Frequency in PTZ-Kindled Rats

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Objective: This study evaluated the anticonvulsant action of lacosamide (LCS), a novel drug that was recently approved for the treatment of partial or secondarily generalized seizures, using an animal model of generalized epilepsy induced by repetitive pentylenetetrazole (PTZ) administration in rats. The main goal was to evaluate the behavioral pattern of lacosamide action by classifying seizures according to a modified Racine-scale. Furthermore, the reproducibility of the win-PTZ kindling model of epilepsy, a recently described variant of the standard PTZ-kindling model, was also assessed. **Methods**: Adult male Wistar rats (n=16) were divided into two groups and underwent the win-PTZ-kindling protocol in two independent trials. After finishing the kindling procedure, all animals, which presented stage 5 seizures were tested for the anticonvulsant action of lacosamide at three different doses (3, 10, and 30 mg/kg). **Results**: The maximal severity of seizures decreased and the latency to stage 3-5 seizures increased when the animals were treated with lacosamide at a single dose of 10 mg/kg compared to saline pretreatment ($p < 0.05$), both parameter reflecting an anticonvulsant action of the drug. Unfortunately, the number of stage 3-5 seizures also increased, but not significantly. The win-PTZ kindling model showed an adequate reproducibility between different trials, however, the number of fully kindled rats was lower than previously reported. **Conclusions**: Lacosamide showed a convincing anticonvulsant action in the win-PTZ kindling model of epilepsy by preventing the generalization of seizures. The win-PTZ kindling model was proved to be useful for studying epileptogenesis and the anticonvulsant action of drugs.

Keywords: lacosamide, anticonvulsant, kindling, epilepsy

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Introduction

Lacosamide is a so-called third generation antiepileptic drug (AED), which is claimed to act on voltage-gated sodium channels in a different way than other anticonvulsants have done so far, i.e., by enhancing the slow inactivation of the channels [1,2]. Furthermore, lacosamide was shown to bind to collapsing-response mediator protein 2 (CRMP-2), another unique mechanism of action proposed for this drug, which was not linked to epilepsy before, but since then several hypotheses appeared describing the role of CRMP-2 in neurite outgrowth [3,4].

The initial discovery that compounds having N-acetyl-alanine-N-benzylamide core structure exhibit a remarkable anticonvulsant activity in the maximal electroshock seizure (MES) test dates back in 1985, when Harold Kohn and colleagues synthesized and evaluated over 250 compounds by the Anticonvulsant Screening Project (ASP) [2]. Lacosamide, the (2R)-2-(acetylamino)-N-benzyl-3-methoxy-propanamide, also known as harkoseride, showed a similar potency to that of phenytoin, the ED50 (i.e. the dose at which 50% of animals experience efficacy) being 4.5 mg/kg when administered intraperitoneally in mice and 3.9 mg/kg after p.o. administration in rats. Several other animal models of seizures were used to characterize the anticonvulsant action of lacosamide before admitting it to clinical trials. Based on the algorithm used by the ASP, a compound showing anticonvulsant activity in the MES test was subject to a testing in the subcutaneous pentylenetetrazole (sc PTZ) seizure test. Lacosamide was incapable to reduce the clonic seizures induced by PTZ at doses of 85 mg/kg and 70 mg/kg in both mice and rats, respectively. However, it significantly increased the seizure threshold in the intravenous PTZ test, which involved a continuous infusion of a 0.5% solution of PTZ [5].

Pentylenetetrazole is a frequently used proconvulsant agent, a GABAA receptor antagonist, which induces acute seizures in laboratory animals after s.c. or i.p. administration at high doses (above 70 mg/kg) [6]. But it can also cause anxiety-like behavior at subconvulsive doses (from 15 to 30 mg/kg) [7]. Interestingly, repeated administration of PTZ at subconvulsive doses (between 35 and 40 mg/kg) induces seizures in rats, these seizures showing increasing severity and culminating in generalization [8]. The process is similar to the widely used kindling technique, where seizures are induced by repeated subconvulsive electrical stimulation [9]. Electrical kindling has been used as a chronic model of temporal lobe epilepsy and complex partial seizures, while chemical kindling models have had different purposes depending on the pharmacological nature of the convulsant agent. PTZ-kindling model is commonly used to study epileptogenesis and test the anticonvulsant action of different compounds [10]. It involves repeated administration of PTZ at a subconvulsive dose

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(30-40 mg/kg) every 48 h until achieving the fully-kindled state. Recently, Davoudi et al. demonstrated that a simplified protocol (named by them "win-PTZ model"), which involves a reduced number of PTZ injections according to a specific dosing schedule over a certain number of days, leads to the same results. The similarity between the win-PTZ kindling model and the standard PTZ-kindling was confirmed by the results of a combined behavioral, electrophysiological and molecular biology experiment [11]. Thus, the win-PTZ kindling model can become a more convenient and more preferable approach because it is less labor-intensive than standard PTZ kindling, but further studies are needed to replicate the initial results in independent studies [12].

Therefore, this study evaluated the action of lacosamide on generalized seizure induced by PTZ using the novel approach for kindling protocol. The main objective was to characterize the behavioral pattern of lacosamide action by classifying seizures according to a modified Racine-scale. The second objective was to evaluate the reproducibility of the win-PTZ kindling model by performing two independent experiments.

Methods

Animals

Male adult Wistar rats (n=16) weighing from 350 to 550 g were kept singly in transparent Plexiglas cages in a laboratory with a temperature of 23±2°C and a relative humidity of 60±10% in natural light/dark cycle. Animals were adapted to laboratory conditions for at least 2 days before the experiments. Throughout the experiment, animals had free access to standard chow and tap water. First of all, all animals underwent win-PTZ kindling protocol without other intervention until reaching fully kindled state. After PTZ injections, each animal was put back into the home cage and observed for one hour. In the post-kindling state, the anticonvulsant action of lacosamide was tested after administering a challenge dose PTZ (Figure 1).

All procedures involving animals and their care were conducted after approval by the local ethics committee for animal experimentation (approval no. 34/2016) and conformed to institutional, national and European Union guidelines (Directive 2010/63/EU).

Drug preparation and administration

PTZ (Sigma Aldrich, St. Louis, USA) was dissolved in 0.9% saline in a concentration of 37.5 mg/ml and injected intraperitoneally (i.p.) at a subconvulsive dose of 37.5 mg/kg every alternate day. After each injection of PTZ, the rats were monitored for one hour and the behavioral severity of seizures was rated by two experienced observers according to a modified Racine scale as follows [8]: stage 0 – no response; stage 1 – ear and facial twitching; stage 2 – myoclonic jerks without rearing or convulsive waves through the body; stage 3 – myoclonic jerks with rearing; stage 4 – turn over into side position, clonictonic seizures; stage 5 – turn over into back position, generalized tonicclonic convulsions. An animal was considered fully-kindled when it have had stage 4-5 seizure score on two consecutive trials.

The anticonvulsant action of lacosamide in three different doses was tested on fully-kindled animals only, from either of the two groups. In this phase, each dose of PTZ used for seizure induction was administered on every alternate day for a period of 10 days (days 0, 2, 4, 6, 8 and 10). Lacosamide (3, 10, 30 mg/kg) was dissolved in 0.9% saline and were administered i.p., a single dose 15 minutes before the PTZ injection (according to LCS pharmacokinetics this is the time of peak effect). Lacosamide was granted by Hetero Drugs Ltd., India. The severity and the latency to the onset of seizures were noted. In order to exclude an unexpected modification of seizure pattern as a possible source of confounding only those animals were included which showed stage 5 seizures at the first PTZ challenge dose (T0) administered in the post-kindling period. Furthermore, at the end of the experiment each animal was retested with another challenge dose of PTZ (T10) and those animals which did not have stage 5 seizures were excluded (n=1). A detailed timeline of this experiment can be found in Figure 1.

Statistical analysis

Data were analyzed with GraphPad Prism 5 (GraphPad Software, San Diego, CA). The results are expressed as the mean ± SEM. The maximal seizure severity observed during the kindling period was compared between the groups using a two-way ANOVA test. In the testing period, the analysis of maximal seizure severity and latency to the onset of stage 3-5 seizures was performed with repeated

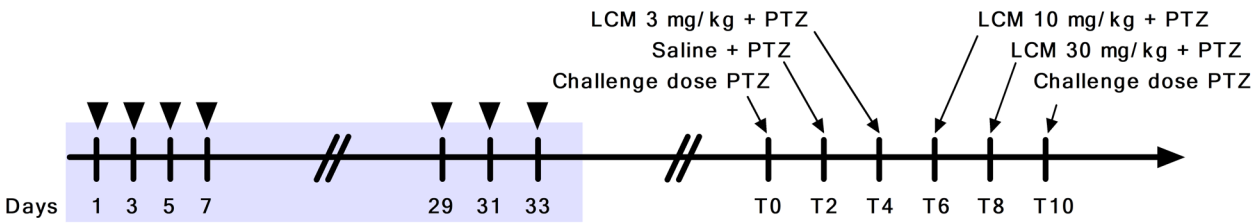

Fig. 1. Schematic timeline of the study design, highlighting the PTZ-kindling procedure and the drug testing in the post-kindling period

Legend: The shaded part of the timeline indicates the win-PTZ kindling procedure, and the double lines crossing the timeline represent latent periods, when no interventions were being performed. Arrowheads show the time of PTZ administration, and arrows show the time of drug or saline administration followed by a challenge dose of PTZ.

measures one-way ANOVA test; the number of stage 3-5 seizures were compared using Friedman test followed by a Dunn's post-hoc analysis. Means were considered to differ significantly if $p<0.05$.

Results

Win-PTZ kindling model

Two independent trials were performed in drug-naïve animals randomly assigned to each group (n=8/group). The kindling procedure had been started after two days of acclimatization of the animals to laboratory conditions. All animals that showed stage 4-5 seizures on two consecutive PTZ administrations at the beginning of the kindling procedure (on days 1, 3, 5, and 7) were excluded from further studies: one animal in the first group and three in the second group. Furthermore, one animal from the first group died before finishing the kindling procedure, having repetitive stage 5 seizures after the third dose of PTZ. After all, the number of animals achieving fully-kindled state, according to the predefined criteria of having stage 4-5 seizures on two consecutive PTZ administrations, was not different between groups (2/6 vs 3/5, $p > 0.05$). The severity of the observed seizures was also similar (Figure 2), and a significant increase with time was detected in both groups ($F(6,63)=2.927$, $p < 0.05$).

Anticonvulsant action of lacosamide

The maximum seizure score and the latency to the onset of generalized seizures reflected a convincing anticonvulsant action of lacosamide in case of 10 and 30 mg/kg dose. Although a decrease of the maximum seizure score (mean ± SEM) from 4.50 ± 0.27 (after saline administration) to 3.90 ± 0.28 was observed even after administering a small dose of 3 mg/kg LCS, a statistically significant difference was observed only at the dose of 10 mg/kg LCS (a decrease to 3.30 ± 0.15, $F(5,47) = 12.37$, $p < 0.001$), a reduction from stage 5 to 3 of the maximal seizure severity being observed in 7 of 8 animals. Interestingly, further increasing the dose to 30 mg/kg did not cause a more evident decrease of the seizure severity compared to the dose of 10 mg/kg (3.6 ± 0.22 vs 3.30 ± 0.15, $p > 0.05$) (Figure 3). Acute LCS administration at a dose of 10 mg/kg also prolonged the latency to the onset of stage 3-5 seizures to a mean ± SEM value of 19.1 ± 4.5 minutes; a significant difference between saline and lacosamide treatment was observed ($F(5,47) = 2.889$, $p < 0.05$; Figure 4).

On the other hand, the number of stage 3-5 seizures increased when the animals were administered LCS. The highest frequency of stage 3-5 seizures was observed at the dose of 10 mg/kg, but the difference between groups did not reach statistical significance (Figure 5). As the number of stage 3-5 seizures showed an up to 3 fold increase after LCS administration, in a few cases, the monitoring period was extended to 90 minutes in order to assure that all relevant seizures were registered. However, all animals exhibited stage 3-5 seizures during the first hour after PTZ challenge only.

Discussions

The PTZ-kindling model of epilepsy has been used for a long time to study the pathophysiology of epileptic syndromes and to test anticonvulsant drugs. In general, the kindling models more closely resemble human epilepsy syndromes and they are capable to reveal the disease modifying potential of drugs [13]. However, they are time-consuming and labor intensive. The time frame of the kindling model cannot be shortened, supposing that the molecular and cellular modifications, which were caused by an initial insult and resulted in an alteration of neuronal excitability, need time to evolve. But, Davoudi et al. observed that during this critical time interval no further interventions (i.e., PTZ injections) are needed for achieving the fully kindled state in rats [11]. They proposed a method, called win-PTZ model, which uses a reduced number of PTZ injections, a minimum of 7 instead of the 17 injections needed in the standard PTZ-kindling protocol. But reducing the number of injections can increase the interindividual het-

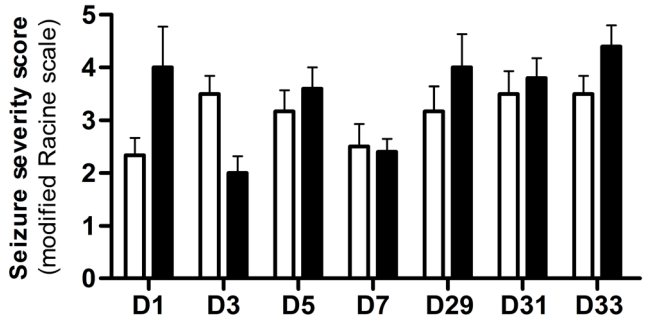

Fig. 2. The evaluation of the reproducibility of win-PTZ kindling model by comparing the intensity of seizures. After comparing the seizure stages between groups and between days using two-way analysis of variance (for repeated measures), there was no significant difference between the groups. Values are expressed as mean ± SEM (n = 8).

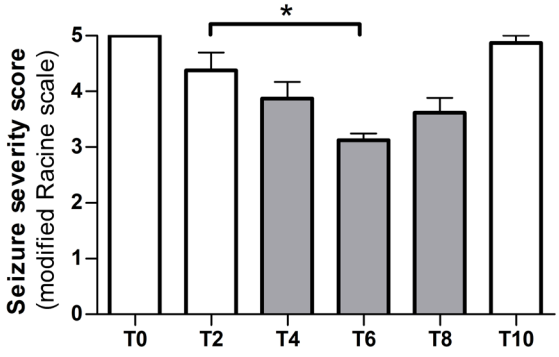

Fig. 3. Lacosamide reduced the maximal seizure severity after acute administration to fully-kindled rats.

Legend: The asterisk shows a statistically significant difference ($p < 0.05$). Values are expressed as mean ± SEM (n = 8). Grey bars indicate lacosamide treatment, and open bars show saline or no treatment. For detailed informations regarding each testing day (T0-T10) see Fig. 1.

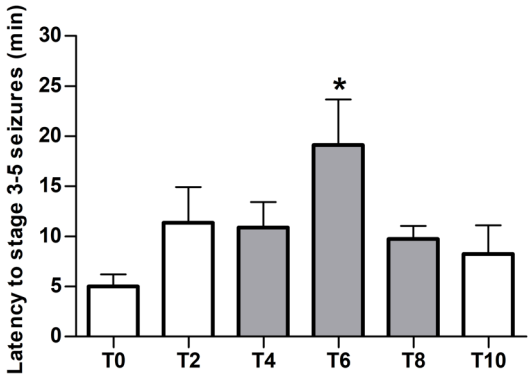

Fig. 4. The latency of the onset of stage 3-5 seizures increased after the administration of lacosamide at a dose of 10 mg/kg.

Legend: The asterisk shows a statistically significant difference ($p < 0.05$). Values are expressed as mean ± SEM (n = 8). Grey bars indicate lacosamide treatment, and open bars show saline or no treatment. For detailed informations see regarding each testing day (T0-T10) see Fig. 1.

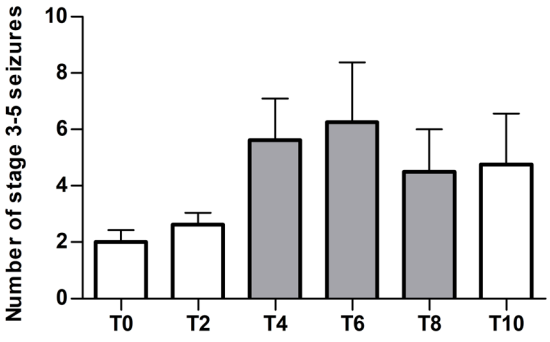

Fig. 5. Lacosamide administration to fully-kindled rats slightly increased the total number of stage 3-5 seizures but without statistically significant differences.

Legend: Values are expressed as mean ± SEM (n = 8). Grey bars indicate lacosamide treatment, and open bars show saline or no treatment. For detailed informations see regarding each testing day (T0-T10) see Fig. 1.

erogeneity in response to the proconvulsant action of PTZ. In this study, two independent trials were performed to reproduce the win-PTZ kindling model. There were no significant differences between them regarding the number of fully kindled rats or the seizure severity. These results support the high reproducibility of the model, however, the number of fully kindled rats observed in this study was lower than that reported earlier. On the other hand, in the post-kindling period, the response of the animals to a challenge dose of PTZ was similar, all animals showing stage 5 seizures.

The anticonvulsant activity of lacosamide was tested on many different seizure models previously. It showed a convincing anticonvulsant effect in MES test and in the 6 Hz model of psychomotor seizure both in rats and mice, the calculated ED50 laying between 5 and 10 mg/kg [5]. However, the acute seizures provoked by s.c. PTZ were resistant to lacosamide up to a huge dose of 100 mg/kg. In kindling models of epilepsy, lacosamide was tested in the rapid hippocampal kindling model and exhibited a dose-dependent reduction in seizure severity, but it required higher doses than for the MES test [2].

In this study, lacosamide demonstrated a remarkable anticonvulsant effect in PTZ kindling model of epilepsy in rats at 10 and 30 mg/kg of dose, significantly decreasing the severity of seizures. In previous studies lacosamide showed no efficacy in PTZ induced seizures, but this study demonstrated that the acute administration of lacosamide reduced the severity of seizures by inhibiting their generalization using the win-PTZ-kindling model. At a dose of 10 mg/kg, lacosamide significantly decreased the severity of seizures. To our best knowledge, this is the first study that tested lacosamide on fully kindled rats in the PTZkindling model of epilepsy in rats.

The main difference between s.c. PTZ induced acute seizures and the PTZ kindling model is the dose of the PTZ used to evoke seizure: in the former case a high dose (85-100 mg/kg) is administered once, whereas in the latter case half of that dose (35-40 mg/kg) is administered repeatedly in order to sensitize the animals to the convulsive effect of PTZ [8,11]. Another important difference between acutely induced seizures by PTZ and PTZ kindling is that sensitization brings cellular and molecular remodeling of neuronal circuits in the brain. It was suggested that the background of the evoked seizures is different: in the early stage of PTZ kindling seizures appear as a result of thalamo-cortical changes, whereas in the late stage the generalized clonictonic seizures originate from limbic structures [10,14]. Thus, in the fully kindling state, the generalized seizures induced by a challenge dose of PTZ resemble seizures of temporal lobe epilepsy in humans, while the acute seizures have cortical origin, a different type of seizure.

Therefore, the anticonvulsant action of drugs in PTZ kindling model differs from that described in the s.c. PTZ seizure model [15]. Taken together, the anticonvulsant effect of lacosamide described in this study is not in contradiction with previously reported results. Presumably, if the type of seizures vary with dose and mode of administration of PTZ, the anticonvulsant action of the tested drugs should also be different.

Lacosamide exhibited a statistically significant anticonvulsant action characterized by a decrease of the seizure severity at 10 mg/kg dose, but interestingly, its efficacy to reduce seizure severity did not increase after administering a dose of 30 mg/kg compared to that observed at 10 mg/kg dose. Unfortunately, lacosamide increased the number of seizures with stage 3 score or above, which suggested an intensification of seizure activity. This observation is in accordance with previously reported data where lacosamide shortened the duration but increased the frequency of epileptiform activities in an in vitro model [16]. Moreover, at 30 mg/kg the latency of stage 3-5 seizures decreased and the maximal severity of the seizures increased, which also suggested that the proconvulsant action of LCS may be

dose-dependent. At this high dose, an increase in seizure susceptibility was described earlier, drug-induced seizures being observed in 50% of the kindled rats treated with 30 mg/kg LCS [17]. Nevertheless, it is commonly accepted that all AEDs can aggravate epilepsy at high, suprathera-peutic doses [18].

Conclusions

Lacosamide, a novel antiepileptic drug, which has recently been approved for use in clinical practice to treat partial and secondarily generalized seizures, demonstrated a remarkable anticonvulsant action in the PTZ kindling model of epilepsy in rats by suppressing the generalization of seizures. However, it seemed to increase the total number of stage 3-5 seizures. Another important finding of this study is that the win-PTZ kindling model of epilepsy, besides its advantages related to ethical aspects and labor intensity of experimental activities, has an adequate reproducibility and is suitable to study either epileptogenesis or the anticonvulsant action of drugs.

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Conflict of interest

None to declare.

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RESEARCH ARTICLE

Validation of High Performance Liquid Chromatography Methods for Determination of Meloxicam and Tenoxicam from Transdermal Therapeutic Systems

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University of Medicine and Pharmacy from Tirgu Mures, Romania

Objective: The aim of this study was to develop and validate two HPLC methods for the quantification of meloxicam and tenoxicam from transdermal therapeutic systems. **Methods**: Based on 1.0% hydroxypropyl methylcellulose 15000, transdermal patches containing meloxicam or tenoxicam were prepared by solvent evaporation technique. Analytical performances of the HPLC methods for the quantification of meloxicam and tenoxicam from such systems were assessed in terms of specificity, linearity, detection limit, quantification limit, recovery and precision. **Results and discussion**: The linearity of the method was assessed through a calibration curve in the 1.0 - 75.0 μg·mL-¹ concentration range, with a regression coefficient higher than 0.999. The detection limit and the quantification limit were found to be 0.46 μg·mL-¹ and 1.39 μg·mL-¹, for meloxicam; and 0.88 μg·mL-¹, respectively 2.64 μg·mL-¹ for tenoxicam. According to the European Pharmacopeia 5.0 the mean recovery was found to be between 75% and 125%. As performance criteria for precision was used the RSD% which were lower than 2.0% for both methods. **Conclusions**: The proposed liquid chromatography methods provide selective, linear and precise results for the quantification of meloxicam and tenoxicam from transdermal therapeutic systems. The presence of a single peak in the chromatograms of the analyzed transdermal patches with meloxicam or tenoxicam, certify the successful determination of the active pharmaceutical ingredient in the prepared patches.

Keywords: meloxicam, tenoxicam, transdermal therapeutic system, high performance liquid chromatography

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) represents one of the oldest classes of therapeutic agents. Despite this, they still are of a great interest in treatment of rheumatic diseases. Conventional pharmaceutical forms may reduce patient compliance by the required multiple administrations. Therefore, the interest in incorporating these drugs into transdermal therapeutic systems (TTSs) has increased in the recent years. Administration of NSAIDs through the transdermal route confers the advantages of maintaining a constant blood concentration of the active ingredient and to reduce the well known gastric side effects [1-3]. Meloxicam (MX), chemically known as 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1, 2-benzothiazine-3-carboxamide-1, 1-dioxide and tenoxicam (TX), chemically known as 4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno (2,3-e)-1,2-thiazine-3-carboxamide-1,1-dioxide, are two substances that belonging to NSAIDs class, which selectively inhibits the enzyme cyclooxygenase-2, being frequently indicated in the treatment of inflammatory diseases [4-6]. Development of transdermal patches involves multiple quality studies for their evaluation [7-11]. Among these types of tests, determining the drug content is one of the most important. The UV spectrophotometrically assay is one of the most common methods used to quantify meloxicam and tenoxicam from TTSs [12-17], mainly because of the low cost. Despite this, a high performance liquid chromatography (HPLC) with ultraviolet detection is more accurate, reproductible and has the advantage of small volume samples.

For this reason, the purpose of this study was to develop two HPLC methods for the quantification of meloxicam and tenoxicam from transdermal therapeutic systems. A validated method can give a real and interpretable information about the analyzed samples, which can generate trusted results.

Methods

Preparation of TTS

Transdermal patches containing 1.3264 mg·cm-² MX (Techno Drugs & Intermediates Ltd. Mumbai, India) or 1.3264 mg·cm-² TX (Nantong Chemding Chephar Co. Ltd. Jiangsu, China) as active pharmaceutical ingredients, were prepared according to a method that has been described in another study [18]. Were acquired TTSs by solvent evaporation technique, with 1.0% hydroxypropyl methylcellulose 15000 (Shin-Etsu Chemical Co., Ltd. Tokyo, Japan). Other substances that were used: propylene

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glycol (Scharlau Chemie, Barcelona, Spain), Tween 20 (Sigma Aldrich Co., France); ultrapure water (Millipore Direct-QS. water distiller), absolute ethanol (Chemical Company, Romania). A placebo patch was prepared as described above without adding the active pharmaceutical ingredient (API).

Validation study

Equipment: HPLC Agilent Technologies 1100 Series (USA) with quaternary pump, degasser, automatic injector, column thermostat, ultraviolet detector and software (Chemstation software); Waters Symmetry C8 column, 4.6x150 mm, 5 μm; analytical balance AB54S (Mettler Toledo, Schweiz); ultrasonic bath T700H (Elma Transsonic).

Chromatographic conditions for MX: mobile phase - phosphate buffer (KH2PO4 20mM, pH 3.0): acetonitrile (60:40 v/v); 1.0 ml/min flow; detection at 362 nm; column temperature was set at 35^{0}C; volume injected 5 μl.

Chromatographic conditions for TX: mobile phase - phosphate buffer (KH2PO4 20mM, pH 3.0): acetonitrile (65:35 v/v); 1.0 ml/min flow; detection at 360 nm; column temperature was set at 35^{0}C; volume injected 5 μl.

HPLC solvents: acetonitrile (Merk, Germany) and methanol (Merk, Germany) with HPLC analytical grade; ultrapure water; the mobile phases were prepared with a mixture of a buffer solution KH2PO4 (Merk, Germany) 20 mM with pH 3 adjusted with phosphoric acid (Merk, Germany).

Analytical performances of the HPLC methods for MX and TX were assessed in terms of specificity, linearity, detection limit, quantification limit, recovery and precision. The calculation of the validation parameters was performed using Microsoft Office Excel 2010 (Microsoft Corporation, USA).

– Method specificity was evaluated by comparing the results acquired for a standard solution of API with a placebo sample. As performance criteria for specificity was used the absence of any interference from excipients in the retention time of the analyte.
– The linearity of the method was assessed through a calibration curve in the 1.0 - 75.0 μg·mL-^{1} concentration range. An initial stock solution of 10 mg·mL-^{1} API was prepared in a volumetric flask with phosphate buffer pH 7.4. This solution was diluted in five different standard solutions with concentrations between 1.0 to 75.0 μg·mL-^{1}. As performance criteria for linearity were used: a linear correlation obtained by plotting the area ratio of API as a function of API concentration and a regression coefficient higher than 0.999 acquired with a least square linear regression analysis. All analyses were performed in triplicate.
– Limit of detection (DL) and limit of quantification (QL) were determined according to the ICH guidelines [19]. Four methods are approached for determining the DL and QL: based on visual evaluation; based on signal-to-noise; based on the standard deviation of the response and the slope; based on the recommended data. In our study the method based on the standard deviation of the response and the slope was approached.

$$DL = (3.3*\sigma)/S \qquad QL = 10*\sigma/S$$

where: σ - was calculated based on the calibration curve (as standard deviation was used the standard deviation of y-intercepts of regression lines); S - slope of the calibration curve.
– Recovery of API was determined in triplicate and was done by comparing the results obtained from API assay from TTS with the results acquired from the standard drug solution with the same concentration. For API assay, TTS samples of 0.7539 cm² were dissolved in a phosphate buffer solution pH 7.4 into a 25 mL volumetric flask, obtaining a final concentration of 40 μg·mL-^{1} of the API. The concentrations of API presented in the samples were determined from the standard curve.
– The precision of the method was analyzed by calculating two parameters: repeatability (intra day precision) and repeatability over different days (intermediate precision). As performance criteria for precision was used the relative standard deviation RSD% which must be lower than 2.0%. The repeatability was done by analyzing in replicate (3 times), three levels of concentration: 50.0 μg·mL-^{1}, 25.0 μg·mL-^{1}, 5 μg·mL-^{1} for MX, and 75.0 μg·mL-^{1}, 50.0 μg·mL-^{1}, 25.0 μg·mL-^{1} for TX, during the same day and under the same experimental conditions. The intermediate precision was done by analyzing sample solutions prepared at the same concentration level, in 3 different days and under the same experimental conditions.

Results and discussion

During methods development and for optimization of chromatographic conditions for quantification of MX and TX from transdermal patches, many compositions of the mobile phase, wavelengths and flow rates were tested. Under the conditions described before, MX showed a characteristic peak at 3.08 min, and the characteristic peak of TX appeared at 3.33 min. Compared with the data reported in the literature [20,21], the retention times of MX and TX have been improved.

– *Specificity*. The results acquired with the placebo patches showed that none of the excipients eluted in the same retention time as the API.
– *Linearity*. Taking into account the peak area response at 362 nm for MX and 360 nm for TX the linearity was observed over the concentration range of 1.0 to 75.0 μg·mL-^{1}. For each drug the correlation coefficient (R^2) was found to be high (0.9999). The analytical curve (Fig.1) data for both API are presented in Table I: slope, intercept and R^2. Statistical data analyze proves that is a linear relationship between the variables (Table I). The chromatograms corresponding to the linearity analyze for MX and TX are shown in Fig.1.
– *Limit of detection*. Limit of quantification. For MX the DL and QL were found to be 0.46 μg·mL-^{1} and 1.39

Table I. Linearity parameters for meloxicam and tenoxicam

Concentration level (µg·mL-1)	Meloxicam				Tenoxicam			
	$A_{Series\ 1}$	$A_{Series\ 2}$	$A_{Series\ 3}$	$A_{Average}$	$A_{Series\ 1}$	$A_{Series\ 2}$	$A_{Series\ 3}$	$A_{Average}$
1	13.2	13.0	13.0	13.1	14.6	14.5	14.1	14.4
5	70.5	70.9	70.8	70.7	68.8	69.7	71.1	69.9
25	348.1	347.5	345.5	347.0	349.8	338.7	340.3	342.9
50	697.2	695.5	696.7	696.5	690.9	693.2	691.3	691.8
75	1049.2	1044.7	1046.5	1046.8	1042.6	1040.3	1041.9	1041.6
Statistical parameters								
Mean equation	Y=13.954x-0.5495				Y=13.875x-0.7939			
Slope	**13.9540**				**13.8750**			
Intercept	**- 0.5495**				**- 0.7939**			
R^2	**0.9999**				**0.9999**			
$^{*}t_{calculated}$	**- 0.92**				**- 0.63**			
$^{*}t_{tabulated}$=2.16	If $t_{calculated} < t_{tabulated}$ (Ordonate at origin does not differ significantly of 0)							
$^{**}C_{calculated}$	**0.657**				**0.67**			
$^{**}C_{tabulated}$=0.68	If $C_{calculated} < C_{tabulated}$ (Determination groups variants are homogeneous)							
$^{***}F_{calculated}$	**3.27**				**2.20**			
$^{***}F_{tabulated}$=3.71	If $F_{calculated} < F_{tabulated}$ (Equation is valid)							

*Student's t test; **Cochran test; ***Fischer test

µg·mL-1 respectively; for TX the DL value was 0.88 µg·mL-1 and the QL was 2.64 µg·mL-1. Similar results were acquired in other studies. For example, in 2009 Mrunalini C. Damle et al obtained for MX a DL value of 219 ng·mL-1 and for QL 722 ng·mL-1 respectively [22]. For quantitative of TX in tablets Singh AK et al [21] obtained as DL a value of 0.35 µg·mL-1 and a QL value of 1.20 µg·mL-1.

– *Recovery*. Using the proposed HPLC method the mean recovery of MX was found to be 97.41±2.02% and for TX it was 87.17±7.43 %. The low recovery of TX may be caused by a experimental error such as a non-homogeneous dispersation of the API during preparation.

A typical chromatograms for API assay are presented in Fig.2.

– *Precision*. The method precision was evaluated in terms

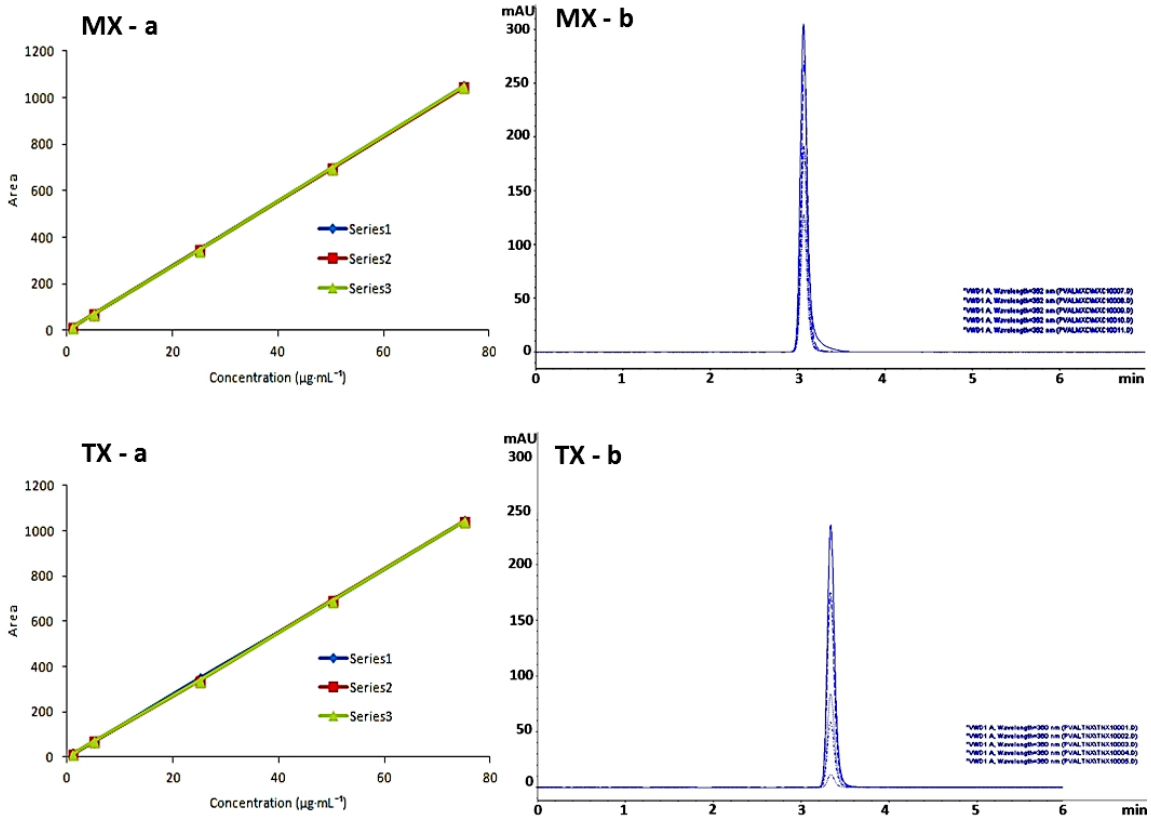

Fig.1. Linearity profiles (a) and chromatograms (b) corresponding to standard solutions of calibration curbes for MX and TX in the 1.0 - 75.0 µg·mL-1 concentration range

Table II. Precision parameters for meloxicam and tenoxicam

Concentration level (µg·mL^{-1})	Repeatability			Intermediate precision		
	Meloxicam					
	50	25	5	50	25	5
	Area - A -					
$A_{Series\ 1}$	701.1	350.5	70.9	697.2	351.1	70.5
$A_{Series\ 2}$	700.5	351.0	70.8	698.3	351.3	70.8
$A_{Series\ 3}$	700.6	351.3	70.9	700.5	350.2	70.9
$A_{Average}$±SD	**700.7±0.3**	**350.9±0.4**	**70.9±0.1**	698.6±1.6	350.8±0.5	70.7±0.2
RSD%	**0.037**	**0.094**	**0.066**	**0.196**	**0.136**	**0.240**
	Retention time (min) - T_R -					
$T_{R\ Series\ 1}$	3.070	3.076	3.077	3.064	3.067	3.069
$T_{R\ Series\ 2}$	3.073	3.076	3.076	3.067	3.069	3.071
$T_{R\ Series\ 3}$	3.070	3.077	3.078	3.069	3.070	3.073
$T_{R\ Average}$±SD	**3.071 ±0.002**	**3.076 ±0.001**	**3.077 ±0.001**	**3.067 ±0.002**	**3.069 ±0.001**	**3.071 ±0.002**
RSD%	**0.046**	**0.015**	**0.026**	**0.067**	**0.040**	**0.053**
Concentration level (µg·mL^{-1})	Tenoxicam					
	75	50	25	75	50	25
	Area - A -					
$A_{Series\ 1}$	1041.9	691.3	253.9	1042.6	693.2	253.9
$A_{Series\ 2}$	1041.2	690.8	253.8	1040.3	691.3	254.3
$A_{Series\ 3}$	1040.9	690.9	254.1	1043.2	690.9	254.7
$A_{Average}$±SD	**1041.3±0.5**	**691.0±0.2**	**253.9±0.1**	**1042.0±1.5**	**691.8±1.2**	**254.3±0.4**
RSD%	**0.040**	**0.031**	**0.049**	**0.119**	**0.145**	**0.128**
	Retention time (min) - T_R -					
$T_{R\ Series\ 1}$	3.337	3.335	3.336	3.336	3.335	3.335
$T_{R\ Series\ 2}$	3.335	3.335	3.336	3.337	3.336	3.337
$T_{R\ Series\ 3}$	3.336	3.337	3.335	3.339	3.337	3.338
$T_{R\ Average}$±SD	**3.336 ±0.001**	**3.336 ±0.001**	**3.336 ±0.001**	**3.337 ±0.002**	**3.336 ±0.001**	**3.337 ±0.002**
RSD%	**0.024**	**0.028**	**0.014**	**0.037**	**0.024**	**0.037**

of inter- and intra-day repeatability and was expresses as RSD %. As shown in Table II, RSD% was found to be below the set 2.0% for all control samples. RSD% ranged from 0.037% to 0.240% and 0.031% to 0.145% for all three levels of MX concentrations and TX, respectively.

Conclusions

In this study two efficient and simple HPLC methods were developed and validated for the analysis of MX and TX in TTS. Based on the current study and on the statistical data, the proposed liquid chromatography methods provide selective, linear and precise results for the quantification of MX and TX from TTS. An advantage of the methods used is the short duration of the API assay (TR for MX: 3.08 min; TR for TX: 3.33 min). The presence of a single peak in the chromatograms of the analyzed TTS with MX and of the TTS with TX, certify the successful determination of the API in the prepared TTS. From this point of view this method can be used successfully to determine MX and TX in TTS.

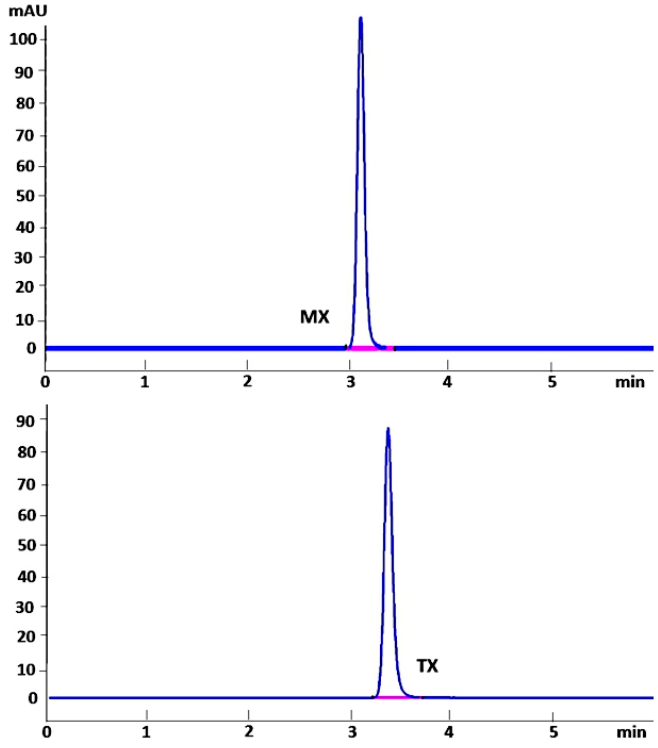

Fig.2. Typical chromatograms for API assay

Acknowledgments

The determinations have been performed in the Drug Testing Laboratory of University of Medicine and Pharmacy of Tîrgu Mureş (https://erris.gov.ro/Drug-Testing-Laboratory), using for data interpretation a special spreadsheet created in Excel (software Microsoft Excel).

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Conflict of interest

None to declare

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RESEARCH ARTICLE

The Relation of Dysfunctional Cognitive Schemas and Personality Dimensions in Generalized Anxiety Disorder

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Objective: This study investigated whether differences exist in the structural personality dimensions and eighteen maladaptive cognitive schemas among in- and out-patients (Clinical Group) diagnosed with Generalized Anxiety Disorder (GAD) and people from the general population without any psychiatric diagnostic (Control Group).

Methods: The Generalized Anxiety Disorder (GAD) sample (N = 100) included 35 (35%) men and 65 (65%) women, with a mean age of 36.4 years (SD = 10.86; age range 18-69). The control sample (N = 100), included 28 (28%) men and 72 (72%) women, with a mean age of 27.1 years (SD = 9.8; age range 19-60). Data were simultaneously analyzed with one-way multivariate analysis of covariance (MANCOVA) to measure the effect of group membership on personality dimensions and on dysfunctional cognitive schemas, controlling for participants' age. Next, univariate analyses of covariance (ANCOVAs) were done on each item with covariate-adjusted post-hoc comparisons.

Results: The results indicate that the Clinical Group participants had unfavorable scores on all five personality dimensions (i.e., Openness, Extraversion; Conscientiousness; Agreeability; and Emotional Stability - Neuroticism) and for almost all of the dysfunctional cognitive schemas in comparison with participants in the Control Group.

Conclusions: These results have general implications in understanding Generalized Anxiety Disorder (GAD) clients and their personality characteristic's profile and dysfunctional/maladaptive cognitive schemas.

Keywords: generalized anxiety disorder, five factor model personality dimensions, maladaptive cognitive schemas, schema therapy, chronic anxiety

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Introduction

Generalized Anxiety Disorder (GAD) is defined by the DSM-5 as the presence of anxiety and worry for at least 6 months, especially related to events and activities in the family, professional, and/or academic area. Accordingly, these individuals find it difficult to control their preoccupation and worry (1). Knowing that GAD severely impacts social and psychological functioning (2), the present study primarily sought to investigate whether there were specific patterns of personality dimensions and maladaptive (dysfunctional) cognitive schemas in patients with GAD. Importantly, as far as we are aware, the present study is the only study that has measured the maladaptive cognitive schemas in patients with GAD with no other comorbidities (e.g., substance abuse, depression, etc.) By determining these specific patterns with the GAD profile, practitioners could improve psychological treatments for GAD. The lifetime prevalence of GAD in a USA nationally representative sample was 5.7%, and prevalence for a 12-month period was 3.1% (3). Epidemiologic studies indicate that lifetime prevalence of GAD for a 12-month period it varies between 1.7% and 3.4% (4). Regarding pathogeny, from a neuro-cognitive point of view, it is a known fact that GAD is associated with hypoactivation in the prefrontal cortex (PFC), which in turn is responsible for emotional regulation that leads to difficulties in the control of emotions, especially worry (5-6).

The Five Factor Model

The Five Factor Model (FFM) defines personality by the way a person relates to his own past life experiences and consists of five personality factors, namely *Openness*, *Extraversion*, *Conscientiousness*, *Agreeability,* and *Emotional Stability Neuroticism* (7-11). Out of these five personality dimensions, *Extraversion* and *Neuroticism* are predictors of anxiety and other affective/psychological disorders (12-14). Further, *Conscientiousness* is positively correlated with Generalized Anxiety Disorder (15). However, the one personality factor that best indicates the presence of anxiety/depression is a low level in the *Emotional Stability* dimension (16). Moreover, having a high level of *Agreeability* and a low level of *Emotional Stability* indicates the presence of anxiety (17).

Maladaptive Cognitive Schemas

It has been shown that maladaptive cognitive schemas (MCS) develop in early childhood, emerging from negative interpersonal interactions or relationships within various environments, such as family, school, and/or networks

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of friends. As a consequence, dysfunctional schemas will become manifest in adulthood, when the patient experiences life situations similar to those experienced during childhood (18-19). These dysfunctional cognitive schemas (see Table 1) greatly influence the way individuals think, feel, and behave in various social environments. Similarly, the CBT model explains the etiopathogenesis of GAD as being facilitated by learning processes which could explain why emotions and interpersonal relationships are a constant source of perceived danger for GAD patients (20).

Objectives

The primary aim of this study was twofold. First, this study sought to investigate whether there are differences in the structural personality dimensions, as defined by the Five-Factor Model of personality (i.e., Openness, Extraversion, Conscientiousness, Agreeability, and Emotional Stability – Neuroticism) among in- and out-patients diagnosed with Generalized Anxiety Disorder (GAD) and people without any psychiatric diagnosis from the general population. The second objective of the study was to identity whether there are differences in eighteen maladaptive cognitive schemas (21) (i.e., Emotional Deprivation, Abandonment/Instability, Mistrust/Abuse, Social Isolation/Alienation, Defectiveness/Shame, Failure, Dependence/Incompetence, Vulnerability to Harm or Illness, Enmeshment/Undeveloped Self, Entitlement/Grandiosity, Insufficient Self-Control/Self-Discipline, Subjugation, Self-Sacrifice, Approval-Seeking/Recognition-Seeking, Negativity/Pessimism, Emotional Inhibition, Unrelenting Standards/Hypercriticalness, and Punitiveness) among patients diagnosed with GAD and people without any psychiatric diagnosis.

Methods

Compliance with Ethical Standards

Written consent for research purposes was obtained from participants after they received the information about the study, and from appropriate Romanian authorities (Ethics Commission of Research from UMF Tirgu Mures). Participants' privacy was protected by replacing their names with identification numbers on all research documents and analyses. This study meets the clinical criteria developed by the International Conference on Harmonisation – Good Clinical Practice (22) and ethical principles and code contained in Nuremberg (23).

Participants and Procedure

The sample consisted of clinical (diagnosed with GAD) and non-clinical adult. In the combined sample (N = 200), the mean age at the time of interview was 31.7 years (SD = 11.4), ranging from 18 to 69 years. Out of 200 par-

Table 1. A Description of Each Maladaptive Cognitive Schema as Measured by the YSQ-S3 Scale adapted for Romanian Population (Young, 2005; Trip, 2006)

YSQ-S3 Scale	Description
Emotional Deprivation	The individual thinks others are depriving him/her of the emotional support he/she needs, not offering protection and understanding.
Abandonment/Instability	The individual's belief that other people will not be able to offer him/her emotional support due to their own instability, absence, or possibility to leave them for someone else.
Distrust/Abuse	The subject's belief that others will eventually take advantage of him/her, intentionally deceiving or hurting, humiliating, lying, or causing pain.
Social Isolation/Alienation	The subject's feeling of social isolation; the feeling that he/she is different from the others, thus being rejected from a social group.
Defectiveness/Shame	A schema which refers to an inferiority complex; the subject seeing himself/herself as incapable, worthless, a bad person, as well fearful of no longer being loved when others notice these things.
Failure	The subject's conviction that he/she cannot correctly perform most actions, as well as the feeling that no matter how hard they try, failure in the important areas of life is certain.
Dependence/Incompetence	The sensation of being incapable of attaining daily goals and objectives without help and supervision from others; the individual feels helpless.
Vulnerability to Harm/Illness	The exaggerated fear of imminent catastrophes which can occur at any time and which cannot be prevented.
Enmeshment/Undeveloped Self	The subject's exaggerated need to be close to relevant people in one's proximity (e.g. parents), thus involving an exaggerated emotional relationship, leading to a certain degree of dependence and underdevelopment of the subject's autonomy and social identity.
Entitlement/Grandiosity	The subject's belief that he/she is superior to others, thus deserving special rights and privileges. A disproportionate sense of superiority is revealed, the goal being to gain control and power.
Insufficient Self-Control	Low tolerance for frustration, lack of impulse control, difficulty controlling oneself, lack of discipline in order to reach certain goals, emotional instability, a disproportionate need to maintain comfort.
Subjugation	The subject aims to gain complete control over others.
Self-Sacrifice	An exaggerated focus on satisfying other people's daily needs, for the purpose of avoiding being seen as selfish by others, while at the same time wishing to maintain a relationship with the person the individual wishes to help.
Approval Seeking/ Recognition-Seeking	The subject's exaggerated need to obtain others' approval and recognition, so that one's self-esteem becomes dependent on other people's reactions.
Negativity/Pessimism	The subject enhances the negative and pessimistic aspects of life, minimizing the positive and optimistic ones.
Emotional Inhibition	The subject represses his/her actions, feelings, and spontaneous communication in order to avoid disapproval or a feeling of shame.
Unrealistic Standards/ Hypercriticalness	The subject's belief in perfectionism and completeness in reaching one's goals, based on rigid "must"-like assumptions and extremely high performance standards, with the purpose of avoiding criticism
Punitiveness	The subject's belief that people who do not satisfy his/her own standards should be punished for their "mistakes". The subject is also critical with one's own person and finds it difficult to forget the errors of others.

ticipants, 137 (68.5%) were female and 63 (31.5%) were male; 176 (88%) were Romanian and 24 (12%) of other ethnic origins. There were no significant differences (α = .05) regarding gender and level of education between the subjects in our GAD and control samples. Additionally, participants in the GAD group were statistically significantly older (M = 36.40, SD = 10.96) than their counterparts in the Control group (M = 27.05, SD = 9.84), t (195.75) = 6.35, p < .001.

The GAD group (N = 100) consisted of 35 (35%) men and 65 (65%) women, with a mean age of 36.4 years (SD = 10.86; age range 18–69). Of these patients, 61(61%) had graduated from college or had postgraduate education, while the remaining 39 participants (39%) were educated at lower levels. In addition, 80 participants in this sample (80%) were Romanian and 20 (20%) of other ethnic origins. The Control group (N = 100), representing a non-clinical population, was composed of 28 (28%) men and 72 (72%) women, with a mean age of 27.1 years (SD = 9.8; age range 19–60). Within this sample, 66 participants (66%) had graduated from college or had postgraduate education, while the remaining 34 participants (34%) were educated at lower levels. In addition, 96 participants in this sample (96%) were Romanian and only 4 (4%) had other ethnic origins. Most of participants in this group (68%) were enrolled in undergraduate medical courses at the University of Medicine and Pharmacy of Tirgu-Mures, Romania; and the rest of the participants had various other professions.

All participants filled out paper-pencil questionnaires (i.e., DECAS Personality Inventory – DECAS, The Young Schema Questionnaire – Short 3 – YSQ-S3) and at a later date the information was entered into electronic format. The information was collected in the Mures County Clinical Hospital (Psychiatric Clinics No. II), Mental Health Center Tirgu Mures, as well as within two private psychotherapy practices in Tirgu Mures, Romania. The inclusion and exclusion criteria were straightforward. Specifically, for the Clinical group, the inclusion criteria were the presence of Generalized Anxiety Disorder (GAD) diagnosed by a psychiatry specialist, and the exclusion criteria was the presence of (a) psychotic symptoms, (b) severe personality disorders (e.g., borderline, schizoid, paranoid according to DSM-IV), and (c) mixed personality disorders. In addition, for the Control group, the inclusion criteria were the lack of psychiatric pathology, and the exclusion criteria was the presence of (a) anxiety/depression symptoms and/or (b) personality disorders.

Measures

All participants in the Clinical sample were diagnosed with Generalized Anxiety Disorder using structured interviews conducted by seven psychiatrists with vast experience in the field (M = 14.5 years), in conformity with diagnostic criteria for GAD.

Questionnaire

Personality. The DECAS Personality Inventory (24) is a psychometric evaluation instrument that has been calibrated, standardized, and validated on the Romanian population, to assess dimensional spheres of personality The internal consistency of the scales ranges from .70 to .82, and the six weeks' test-retest stability coefficients range from .79 to .91 (25). There is good concurrent validity between the DECAS Personality Inventory and the NEO PI-R Personality Inventory (24), the levels ranging between .57 and .81.

Maladaptive cognitive schemas: The Young Schema Questionnaire – Short 3 (YSQ-S3) is an instrument designed for measuring maladaptive cognitive schemas and it was calibrated, standardized, and validated on the Romanian population. YSQ-S3 consists of 114 items and measures 18 dysfunctional cognitive schemas; The YSQ-S3 subscales have a very good reliability, with Cronbach's alpha coefficients ranging from .68 to .96, and good discriminative validity (26).

Statistical Analyses

For completing the statistical analyses, SPSS (version 19.0) and JASP (version 0.7.5.5) were used for the current research (27-28).

Results

Personality

Importantly, the demographic analyses showed that participants in the GAD group were statistically significantly older than their counterparts in the Non-Clinical group. Considering this result, for measuring the effect of group membership (Non-Clinical vs. Clinical) on all five personality dimensions as defined by the Five-Factor Model (i.e., Openness, Extraversion, Conscientiousness, Agreeableness, and Emotional Stability), and controlling for participant's age as a covariate, a one-way MANCOVA was performed. The results showed a significant multivariate main effect for group membership, Pillai's Trace = .211, F(5, 193) = 10.29, p < .001, but not a significant main effect for participants' age, Pillai's Trace = .004, F(5, 193) = 1.36, p = .984. Significant overall MANCOVA were followed by ANCOVAs with each personality dimension as a dependent variable, group membership as an independent variable, controlling for participant's age (covariate), followed by subsequent post hoc comparisons. As shown in Table 2, these subsequent analyses revealed a significant effect (p <.05, p <.01, and p <.001) of group membership on personality dimensions controlling for participants' age. The effect of group membership had only a marginally significant effect on Conscientiousness (p = .075), controlling for participant's age. Importantly, the covariate (participants' age) was not significantly related to any of the dependent variables (personality dimensions).

Table 2. One-way Analyses of Covariance (ANCOVAs) for DECAS Scale Showing the Difference between Clinical and Non-Clinical Groups (N = 200), and controlling for participant's age

	Non-Clinical Group		Clinical Group				
DECAS Scale	M[a]	SD	M[a]	SD	F[b]	p	η_p^2[c]
Openness	52.43	9.91	48.00	11.45	7.06	<.01**	.035
Extraversion	49.72	12.08	42.08	13.53	14.66	<.001***	.069
Conscientiousness	48.41	10.39	45.56	10.12	3.21	.075	.016
Agreeableness	52.45	8.98	48.89	9.91	5.87	.016*	.029
Emotional Stability	48.20	8.84	39.55	8.19	42.61	<.001***	.178

Note. * p < .05, two-tailed. ** p < .01, two-tailed. *** p < .001, two-tailed.
[a] Estimated marginal means controlled for the effect of participant's age.
[b] ANOVAs df = 1, 197 of group membership .
[c] Effect size (Partial Eta Squared) for differences between groups.

Maladaptive Cognitive Schemas

For measuring the effect of group membership (Non-Clinical vs. Clinical) on all 18 dysfunctional cognitive schemas, and controlling for participant's age as a covariate, a one-way MANCOVA was performed. The results showed a significant multivariate main effect for group membership, Pillai's Trace = .966, $F(18, 180) = 280.59$, $p < .001$, and for participants' age, Pillai's Trace = .196, $F(18, 180) = 2.43$, $p < .01$. Significant overall MANCOVA were followed by ANCOVAs for each dysfunctional cognitive schema as a dependent variable, group membership as an independent variable controlling for participant's age (covariate) followed by subsequent post hoc comparisons. These subsequent analyses revealed a significant effect ($p < .05$ and $p < .001$), of group membership on maladaptive cognitive schemas controlling for participants' age. Specifically, as shown in Table 3, participants in the Clinical group had significantly higher scores on 16 dysfunctional cognitive schemas but not on Emotional Inhibition and Unrealistic Standards scales compared with their counterparts, controlling for participants' age. Importantly, the covariate (participants' age) was significantly related to dysfunctional cognitive schemas only in the case of Distrust/Abuse; Social Isolation/Alienation; Entitlement/Grandiosity; Self-Sacrifice; and Emotional Inhibition.

Discussion

As hypothesized, the results of the present study indicate that participants in the Clinical group (i.e., patients diagnosed with GAD with no comorbidities) had a higher level of unfavorable scores on all five dimensions of personality (i.e., Openness, Extraversion, Conscientiousness, Agreeability, and Emotional Stability – Neuroticism) than participants without any psychiatric diagnosis, controlling for participants' age. In addition, the results also showed that participants in the Clinical group had a higher level of unfavorable scores on all dysfunctional cognitive schemas except *Emotional Inhibition* and *Unrealistic Standards*

Table 3. One-way Analyses of Covariance (ANCOVAs) for YSQ-S3 Scale Showing the Difference between Clinical and Non-Clinical Groups (N = 200), and controlling for participant's age

	Non-Clinical Group		Clinical Group				
YSQ-S3 Scale	M[a]	SD	M[a]	SD	F[b]	p	η_p^2[c]
Emotional Deprivation	11.80	5.12	21.04	3.13	196.07	<.001***	.499
Abandonment/Instability	13.14	5.37	22.92	2.21	234.06	<.001***	.543
Distrust/Abuse†	14.41	5.23	23.50	2.93	198.69	<.001***	.502
Social Isolation/Alienation†	14.01	4.75	15.71	4.27	5.97	<.05*	.029
Defectiveness/Shame	8.48	4.21	16.84	3.23	205.91	<.001***	.511
Failure	12.78	5.71	18.73	3.05	70.50	<.001***	.264
Dependence/Incompetence	13.73	5.42	18.14	3.32	39.95	<.001***	.169
Vulnerability to Harm/Illness	10.34	4.20	18.48	2.28	240.54	<.001***	.550
Enmeshment	10.71	4.89	18.07	3.75	117.99	<.001***	.375
Entitlement/Grandiosity†	10.10	5.16	21.44	2.27	368.94	<.001***	.652
Insufficient Self-Control	15.18	7.09	20.14	2.67	36.01	<.001***	.175
Subjugation	12.61	5.18	18.96	2.54	101.84	<.001***	.341
Self-Sacrifice†	14.25	6.20	19.46	4.08	42.74	<.001***	.178
Approval Seeking	29.92	9.39	58.10	12.48	271.61	<.001***	.580
Negativity/Pessimism	14.00	6.05	46.35	5.79	1237.09	<.001***	.863
Emotional Inhibition†	33.51	13.16	21.36	3.16	70.68	<.001***	.264
Unrealistic Standards	30.84	9.42	20.41	2.39	95.87	<.001***	.327
Punitiveness	38.39	11.29	59.54	8.03	193.45	<.001***	.495

Note. * <.05, *** p < .001, two-tailed.
[a] Estimated marginal means controlled for the effect of participant's age.
[b] ANOVAs df = 1, 197 of group membership.
[c] Effect size (Partial Eta Squared) for differences between groups. †For this item, the participant's age also had a statistically significant effect.

when compared with participants in the Control group (non-clinical participants).

Specifically, (see Table 2) our study revealed that patients with GAD showed statistically significantly lower scores on *Openness* (*M* = 48.00), *Extraversion* (*M* = 42.08), *Conscientiousness* (*M* = 45.56), *Agreeableness* (*M* = 48.89), and *Emotional Stability* (*M* = 39.55; or high *Neuroticism*), controlling for participants' age, compared with their counterparts in the Control group. In the case of *Conscientiousness* (*M* = 45.56), the difference was only marginally significant. This pattern of results is consistent with other studies which found low scores on these dimensions of personality in clinical samples. Specifically, these studies not only showed low scores on *Extraversion*, *Conscientiousness*, and *Emotional Stability* (high *Neuroticism*), but also they revealed a relationship between these personality dimensions and the presence of anxiety and depression symptomatology (29-30). Not only are the aforementioned low scores of these personality dimensions associated with anxiety and depression symptomatology, but along with low *Agreeableness*, these four characteristics are typically associated with almost all types of symptoms of clinical disorders (31). Furthermore, in the case of GAD patients, a general personality profile highlights the presence of low *Extraversion* and *Emotional Stability* represented by various psycho-behavioral patterns such as shyness, lack of social enthusiasm, avoidance to take on leadership roles, emotional repression, social inhibitions, distress, feelings of hopelessness and helplessness, as well as pessimism (17). Our results suggest that the chronic nature of GAD could be attributed to personality because the dimensional structure of personality is stable, with certain alterations occurring over time only at the surface level (11). Also, high *Neuroticism* plays an important role in the occurrence and persistence of the specific worrying symptoms of GAD, even if the two (Neuroticism and GAD) are different clinical entities (32). In addition, low scores of *Agreeability* and *Emotional Stability* were also found in a non-clinical sample of undergraduate students (33), where these two personality dimensions were also associated with maladaptive cognitive schemas, supporting the assumption that, in general, personality dimension can be seen as a predictor of these dysfunctional patterns of thinking.

In our study, out of the 18 maladaptive cognitive schemas (MCS/dysfunctional schema), 16 were statistically significantly higher (unfavorable) for patients with GAD compared with participants with no psychiatric pathology, controlling for participants' age (see Table 3). Two schemas were an exception, occurring at a higher level in the patients with no psychiatric pathology, namely the *Emotional Inhibition* and the *Unrealistic Standards* schemas. Specifically, the MCS which occurred at the highest levels in GAD patients were *Emotional Deprivation* (*M* = 21.04), *Abandonment/Instability* (*M* = 22.92), *Approval Seeking* (*M* = 58.10), *Negativity/Pessimism* (*M* = 46.35), and *Punitiveness* (*M* = 59.54). Generally, in the case of GAD patients, these MCS are responsible for a dysfunctional thinking pattern. For example, *Emotional Deprivation* is often exhibited as a dysfunctional thinking pattern where an individual with GAD worries about the likelihood of loved ones being unable to offer the emotional support and safety they need. Moreover, there are cognitive distortions generated by the *Abandonment/Instability* schema, which causes feelings of intolerance to uncertainty, characterized by the fear of being deserted for another person or by worry related to the fact that emotional support from a loved one in a time of need might not be received. In this context, the *Approval Seeking* schema generates a relationship with the world and those around the individual that is formed by way of feedback received from the people around him or her, so that self-esteem becomes dependent on the reaction of others. *Punitiveness* is responsible for a critical perception of one's own person as well as of those around him or her, so that errors committed by others, or by oneself, are forgotten with difficulty.

MCS are not only associated with GAD, but also are related with anxiety/depression disorders, and the presence of these schemas can also indicate comorbidities with various personality disorders or substance abuse (34). Furthermore, there are specific MCS associated with each personality pathology (35). We know that GAD is strongly associated with personality disorders (36), and from a clinical and comorbidity point of view, GAD is also similar to emotional disorders, namely depression (18). For instance, regarding depressive symptoms, an association has been found between high *Neuroticism* and low *Extraversion*, and the presence of MCS (37). Two schemas are mainly involved here, namely *Emotional Deprivation* and *Abandonment/Instability* (38). Interestingly, these two schemas are similar both in GAD patients and depressive patients; and it would be interesting to find out in future studies whether both GAD and depressive patients present common dysfunctional cognitive schemas.

Conclusions

The results of this study have general implications regarding our understanding of GAD clients and their personality profile characteristics and maladaptive cognitive schemas. Additionally, these results encourage us to believe that cognitive-behavioral therapy (CBT) intervention for GAD patients could be adapted to target the specific symptomatology of this disorder such as (a) the maladaptive cognitive schemas that play a role in the chronic nature of GAD, and (b) the disharmonic dimensional structure of these patients' specific personalities.

The intensity and severity of MCS in anxiety disorders or chronic psychological conditions (including GAD) may indicate the use of a complex psychotherapeutic approach such as Schema Therapy (ST). This is due to the fact that patients with high MCS scores present a primary diagnosis of anxiety disorder, which can co-occur with personality disorders or organic illnesses. Specifically, ST is

a therapeutic direction focused on persistent or chronic psychological issues and may be appropriate in the context of GAD (39). Furthermore, treating GAD through Metacognitive Therapy (MCT) can also be a viable treatment option, based on the results presented in this study. For this purpose, however, the conceptualization of the MCS-based model (specific to ST) should be transitioned to a more MCT specific model. Moreover, an integrative approach of CBT, combining multiple techniques or orientations (i.e., Rational Emotive Behavior Therapy, Cognitive Therapy, Schema Therapy or Metacognitive Therapy, and Mindfulness) can also be a good therapeutic approach for GAD (20).

Thus, future studies should investigate the impact of treatment, comparing Schema Therapy with antidepressant medication in treating GAD. It would be interesting to determine whether the efficiency of a direct approach to MCS's through Schema Therapy is superior, equal, or inferior to antidepressant medication. Based on the present study, a specific therapeutic plan for treating GAD could be created, tested, and empirically validated using the findings from this study.

Limitations

Non-probability sampling techniques were used in selecting the participants of the current study, and, consequently, caution is necessary when interpreting and generalizing the outcomes of this convenience sample study. Furthermore, the self-reported measures (i.e., DECAS Personality Inventory and Young Schema Questionnaire – Short 3) could have been influenced by social desirability bias or the presence of psychopathologic symptomatology. In addition, the present study only investigated the differences between clinical and non-clinical groups across variables for personality characteristics and maladaptive cognitive schemas, and, therefore, no causal relationship can be assumed. Additionally, the significant effect of participants' age on five schemas (i.e., Distrust/Abuse; Social Isolation/Alienation; Entitlement/Grandiosity; Self-Sacrifice, and Emotional Inhibition) should be taken into account when results are interpreted. Future studies (with a more diverse sample in respect to age) are needed to elucidate the nature of this relationship.

Conflict of interest

None to declare.

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CASE REPORT

Simultaneous Extrahepatic Portal and Iliac Veins Thrombosis After Abdominal Surgery - A Case Report and Review of Literature

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Introduction: Extrahepatic portal vein thrombosis (EPVT) is the most frequent cause that leads to portal hypertension in non-cirrhotic patients. This condition is related to systemic and local risk factors (such as inflammatory lesions, injuries to portal venous system by surgery, vascular procedures). **Case presentation**: A case of extended extrahepatic portal vein thrombosis and simultaneous thrombosis of left common iliac vein and inferior vena cava, appeared after abdominal surgery in a hypertensive, diabetic, 50 y.o. man is presented. An acute episode of abdominal pain was interpreted as an emergency and a surgical (initially laparoscopic and then open) procedure was planned in order to perform an appendectomy. Discharge diagnosis was hemoperitoneum secondary to iatrogenic rupture of sigmoid mesocolon provoked by trocar manipulation. Repeated imaging studies performed later revealed the thrombosis of portal vein with extension into right portal branch associated with superior mesenteric thrombosis and free-floating thrombus into left common iliac vein extended towards inferior vena cava. Surgical manoeuvres are considered as triggers of these thrombotic events. After 4 weeks of parenteral anticoagulation a partial recanalization of thrombi was identified, without bleedings. **Conclusions**: Acute EPVT needs a carefully management. Case is linked to abdominal surgery and requires prolonged anticoagulation related to simultaneous portal and iliac vein thrombosis. Associated conditions (hypertension and diabetes mellitus) must have an appropriate approach. After our knowledge this is the first case published in literature.

Keywords: extrahepatic portal and iliac veins thrombosis, complication of abdominal surgery, anticoagulation, long-term management

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Introduction

Extrahepatic portal vein thrombosis (EPVT) - obstruction of the main portal vein with or without extension into the intrahepatic branches – is considered the most common cause which leads to portal hypertension (PHTN) in case of non-cirrhotic patients. Interruption of the portal vein has few clinical consequences, due to two compensatory mechanisms [1,2].

Local and systemic risk factors are involved in the etiology of EPVT; still in 1/3 of patients a combination of thrombotic risk factors are present. Important local factors are cirrhosis, infection, inflammation (diverticulitis, pancreatitis, cholecystitis, inflammatory bowel disease), and malignancy (hepatocellular, gastric, pancreatic cancer). Systemic factors include both genetic deficiencies affecting coagulation and non-genetic conditions (myeloproliferative disorders, polycythemia, pregnancy, use of oral contraceptives). Postoperative portal vein thrombosis (PVT), although rare, can occur after surgical procedures which require the manipulation of major portomesenteric veins (splenectomy) or after laparoscopic procedures (appendectomy, gallbladder removal, colon or bariatric surgery for morbid obesity) [1,2,3,4,5,6].

Acquired or inherited disorders in the coagulation pathways often provoke PVT. Factor V Leiden deficiency, prothrombin gene G20210A mutations, deficiencies of intrinsic anticoagulation factors are mentioned. Acquired disorders include antithrombin III deficiency, disseminated intravascular coagulation, malnutrition, sepsis, inflammatory bowel disease, liver disease. Rarely, thromboses of the splenic vein has an extension into the portal vein, leading to adjacent inflammatory response (pancreatitis) [7,8,9].

Remarkable, the majority of patients do not present symptoms, while others gradually develop PHTN, varicose veins with a major risk of gastrointestinal massive bleeding.

Spleen enlargement can be observed in rare cases. If EPVT is suspected, non-invasive imaging techniques usually confirm the diagnosis and the presence of solid material into the venous portal lumen [1,2,8,10,11,12].

Case presentation

The main objective of this paper is to present an extremely rare complication of abdominal surgery and the management of a case of extended PVT in a patient with positive family history of hypercoagulable state or thrombotic

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events (father: thrombophlebitis; brother: procoagulant status)**;** secondary objective is to emphasize the importance of an appropriate medical approach in long-term management of such life-threatening cases in family practice.

A 50-year-old caucasian male, hypertensive, type 2 diabetic insulin-dependent, presented to the emergency medical unit in a secondary-care hospital with an acute episode of abdominal pain related to food abuse. A surgical emergency (acute appendicitis) was considered based on the patient complains, physical examination findings, complete blood count (increased white blood cell count with neutrophilia) and positive CRP; other tests (ALAT, ASAT, serum amylase, urinalysis) were negative. Hyperglycaemia (13.6 mmol/l) was interpreted postprandial. No imaging studies were performed and an emergency appendectomy was considered (initially standard laparoscopic converted to open procedure– pararectus incision). Discharge diagnosis was hemoperitoneum secondary to rupture of sigmoid mesocolon, sustained by a decreased hematocrit level to 35 %. No appendectomy was performed. No anticoagulation regimen was used before and after surgery.

As the epigastric pain did not disappear over the next week after surgery, patient was readmitted and a series of medical tests were performed in order to clarify the etiology of pain. Repeated abdominal and lower-veins ultrasound examinations, thoracic-abdominal CT scans and upper digestive tract endoscopy were performed 12 days after surgery and later on, and the suspicion of EPVT was raised.

Patient was referred to a tertiary care hospital, cardiology unit. Upon admission, patient presents normal physical status, blood pressure, pulse and rest EKG recording within physiological limits and normal bowel transit.

Laboratory data including a basic metabolic panel, complete blood count, presented normal values except INR (1.53), using a standard dose of 2 mg Acenocoumarol daily.

Repeated abdominal ultrasound (Philips HD11XE ultrasound machine) and abdominal CT scan (Siemens Somatom Definition CT scanner) with intravenous contrast media (Iomeron 350/100) revealed the presence of (Fig. 1, Fig. 2, Fig.3):

– Thrombosis into the portal vein with extension into the right portal branch associated with superior mesenteric thrombosis and free-floating thrombus into the left common iliac vein extended towards IVC
– Biliary microlithiasis
– Gallbladder hydrops
– Edematous pancreatitis

As malignancy is one of the leading causes of portal thrombosis, a search for tumoral markers was performed, but the results were negative (alpha fetoprotein=1.07 UI/ml, CA 19-9 < 3.0 UI/ml).

Patient was tested for inherited disorders in the coagulation pathways, taking into consideration family history and that factor V Leiden mutation is considered to be the most important thrombophilic predisposing factor for

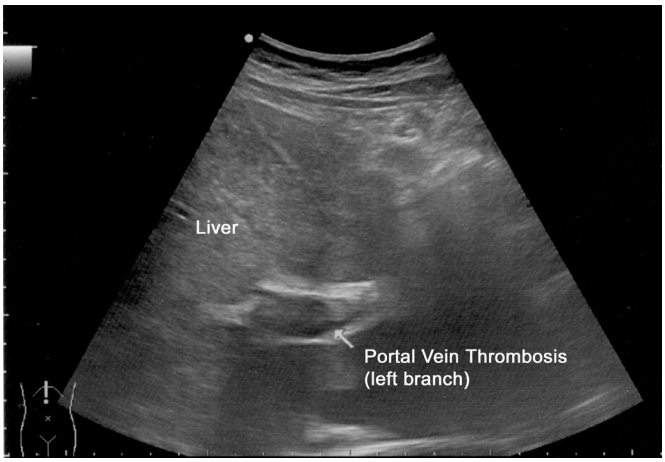

Fig. 1. 2D abdominal ultrasound examination – visualisation of the thrombus in left branch of the portal vein (arrow)

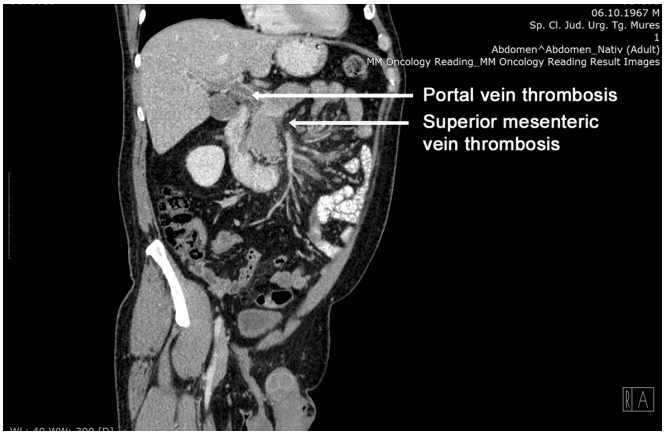

Fig. 2. Contrast-Enhanced abdominal CT examination – visualisation of the thrombus in the portal vein and in the superior mesenteric vein (arrows)

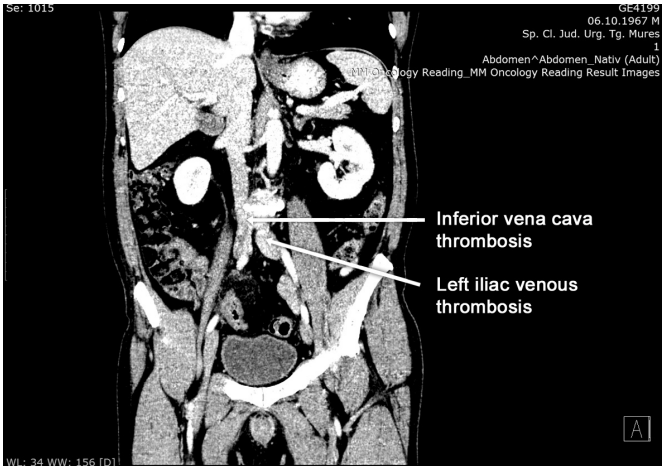

Fig. 3. Contrast-Enhanced abdominal CT examination – visualisation of the thrombus in the inferior vena cava and in the left common iliac vein (arrows)

PVT. Blood samples for a thrombophilic status (protein C and S deficiency, G20210A mutation in the prothrombin gene, factor V Leiden mutation, enhanced factor VII activity, JAK2 V617F mutation, VCORK1 -1639G>A gene single nucleotide polymorphism, PAI-1 4G/5G) were negative.

No other possible causes were identified and thus an acute episode of pancreatitis and extensive surgical ma-

noeuvres were considered as causes of these thrombotic events.

Prolonged parenteral anticoagulation regimen (Enoxaparin 1 mg/kg body weight b.i.d.) was started, associated with PPI (Pantoprazole 40 mg o.d.), non-selective beta-blockers (Propranolol 10 mg t.i.d.), a combination of enzymes (Kreon 10000), and adjusted doses of Insulin.

After 4 weeks of parenteral anticoagulation a partial recanalization of portal and iliac veins was confirmed by imaging studies. At discharge medical counselling was given with ambulatory parenteral anticoagulation with 1 mg/kg body weight b.i.d. Enoxaparin. Patient was scheduled for readmission in 6 weeks for reassessment and switching for oral anticoagulation. Abdominal CT scan with contrast media was performed in order to delineate evolution of extended portal vein thrombosis. A total resolution of the thrombosis from the portal vein and inferior cava vein was observed, while into the common iliac vein was noticed a partial resolution of the thrombus. Oral anticoagulation with adjusted doses of Acenocumarol (for INR: 2.5-3) was started in order to prevent any other thrombotic events. Advices for self-monitoring anticoagulation regimen using a point-of-care device (CoaguCheck® XS Plus Pro System), antihypertensive regimen and self-monitoring of blood sugar levels were offered.

Discussions

PVT usually represents a rare complication of laparoscopic surgery that can be life threatening if it affects the mesenteric venous arches and leads to mesenteric ischemia or infarction [3,5,13].

Etiology of PVT in non-cirrhotic patients is usually divided into local and systemic factors. Genetic coagulopathies that are considered to be associated with PVT such as factor V Leiden, the prothrombin G20210A mutation, protein C and S deficiency, antithrombin III deficiency must be also taken into consideration [1,2,6,11,12,14,15].

Laparoscopic appendectomy may have inherent potential complications. Infection at the surgical sites is the most common associated complication. Other complications include small bowel obstruction, haemorrhage (intraabdominal, hematoma of abdominal wall, scrotal), ileus, stump complications, hernia. In the case of patients with a recent episode of EPVT, the aim of the treatment is to restore flow patency and stop extension of the thrombosis, while in chronic EPVT, portal hypertension management is prior [1,2].

Therapeutic anticoagulation is recommended in stable, non-cirrhotic patients with acute PVT. The recommended duration of treatment is from 6 up to 12 months in order to achieve a stable resolution of thrombosis. Treatment should be provided lifelong in patients known with prothrombotic status, with the goal of maintain a proper recanalization of affected veins [1,5,10,16,17].

Variceal bleeding, the most common and dangerous complication of portal hypertension, can be effectively controlled and prevented using endoscopic therapy [16]. If endoscopic therapy fails to proper manage the bleeding, a transjugular intrahepatic portosystemic shunt, along with another alternative of surgical shunt can be considered [14].

Conclusions

In this particular case, the diagnosis of appendicitis leads to the decision of a laparoscopic appendectomy. Intraabdominal haemorrhage secondary to the rupture of mesocolon during trocar manipulations direct the decision to a classic open surgery. On abdominal exploration, the appendix was not inflamed; no other specific common surgical pathologies (i.e. acute pancreatitis, cholecystitis, tumours, acute mesenteric ischemia, etc.) were identified; surgical haemostasis of mesosigma was performed. Manoeuvres during laparoscopic appendectomy were considered to be the trigger of extended thrombosis.

Computer-tomography examination with contrast is a valuable and early diagnostic tool of portomesenteric and iliac vein and vena cava thrombosis, which offers the possibility to avoid any kind of bowel gangrene or perforation, or pulmonary embolism, which can lead to peritonitis and worsen the patient's state [1,2,3].

Treatment should be patient-centred, taking into consideration the extension of thrombosis in the vascular system and absence/presence of bowel ischemia or infarction [13]. If a thrombophilic status is confirmed, life-long anticoagulation is mandatory and an extensive screening of relatives must be performed. Patients who suffer from non-cirrhotic EPVT can experience new thrombotic events associated with decreased survival, especially if they have a prothrombotic disorder [1,10,18].

Oral anticoagulation treatment (VKA) should be provided long-life for prevention of recurrences or development of chronic thromboembolic pulmonary hypertension, associated with antibiotics in case of infection or systemic inflammatory response syndrome. Anticoagulation usually leads to prevention of new thrombotic events, but the risk of gastrointestinal bleeding should not be neglected.

Conflicts of interest

None declared.

Acknowledgements

Patient informed consent was prior obtained for publishing.

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CASE REPORT

Colorectal Carcinoma in a Patient with Situs Inversus Totalis

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Introduction: Colorectal cancer is one of the most common types of malignant tumors worldwide. In patients with situs abnormalities such as situs inversus totalis or situs ambiguus, the presence of this tumor could be a challenge for the surgeon, especially in cases in which the laparoscopic approach is considered. **Case presentation**: We report the case of a 69-year-old male patient with situs inversus totalis. This particular case of situs inversus totalis was not a classical type because the patient had bilateral bilobed lungs, polysplenia, preduodenal portal vein in association with midgut malrotation. The pathology report after surgery revealed moderately differentiated adenocarcinoma of the sigmoid colon, stage pT3 N1c M1a, liver metastases but without metastases in the eight resected lymph nodes. We compared this rare association of diseases of particular anatomic aspects with other reports in the specialty literature. **Conclusion:** The identification of situs abnormalities or other malformations in patients with resectable colorectal cancer is essential, thus preoperative imaging studies are imperative for a proper surgical management. Colorectal cancer metastasizing patterns in patients with intestinal malrotation need to be further investigated.

Keywords: situs inversus totalis, colorectal cancer, midgut malrotation, polysplenia, bilateral bilobed lungs

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Introduction

Colorectal cancer (CRC) is the third most common type of cancer in males and second in females, which makes it one of the most common causes of death worldwide [1]. Over the past few decades, CRC screening methods improved significantly, hence becoming indispensable, especially for detecting earlier-stage CRC. CRC usually presents less specific or no symptoms at all. The surgical approach is mandatory in all operable stages, hence, in the rare case of a situs abnormality, an accurate imaging evaluation of the anatomy is essential. Situs abnormalities include situs inversus totalis (SIT) and situs ambiguus (SA). SIT is a rare congenital condition, with an incidence of 1:10000 births, in which the arrangement of all internal organs are mirrored, hence a complete transposition of organs [2]. SA (or heterotaxy) is characterized by visceral malposition with the ambiguity of atrial morphology. There are two main categories of SA, double left-sidedness or left-isomerism (polysplenia, bilobed bilaterally lungs) and double right-sidedness or right-isomerism (asplenia, trilobed bilaterally lungs). SA has an incidence of 1:10000 - 1:20000 births [2]. Baso M. et al. have reported a number of 14 associations between SIT and colorectal cancer [3]. In this paper, we report a rare case of sigmoid cancer in a 69-year-old male patient with SIT, polysplenia and intestinal malrotation. The patient signed the informed consent for surgery and the publication of this case before the intervention.

Case presentation

A 69 year-old-male patient was admitted to the department of surgery for weight loss, vague abdominal pain and chronic diarrhoea. Personal history included SIT, primary hypertension, obliterating arteriopathy of the lower limbs. Physical examination revealed nothing remarkable except the heart sounds which were heard on the right side of his chest and the dull percussion sound of the liver on the left side of his abdomen. Laboratory findings revealed mild anemia (hemoglobin 12 mg/dl, hematocrit 39%).

The CT scan revealed situs inversus: the location of the heart on the right side with complete transposition of the heart chambers and the great arteries with normal visceroatrial arrangement, concordant atrioventricular connection and normal emergence of the great arteries (Figure 1). The bilobed lungs were present bilaterally without minor fissure (Figure 2). The suprarenal segment of the inferior vena cava was absent and the azygous vein continuation drained into the superior vena cava. Furthermore, our patient had polysplenia and intestinal malrotation. The portal vein had a preduodenal and prepancreatic course. The ligament of Treitz was present, but the small bowel was situated on the right side of the midline and the colon on the left side of the midline. The superior mesenteric vein was situated on the right side of the superior mesenteric artery. The ileocecal junction was found in the middle of the lower abdomen. There was no fixation of the ascending colon with only one left flexure behind the posterior margin of the liver. The sigmoid colon had the mesocolon on the left part of the pelvis. A moderate circumferential

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thickening of the walls in the sigmoid colon (13 mm thick and 30 mm long), minimal infiltration of the surrounding fat and three perisigmoid lymph nodes up to 6 mm were observed. Two hypovascular metastases were noticed in the liver. Diffuse atherosclerotic disease was present with multiple calcified and noncalcified plaques. After the assessment, the patient underwent the surgical resection of the tumor and liver metastases using the classical procedure with laparotomy, followed by mechanical colorectal anastomosis. An anastomosis fistula occurred, which required reintervention. The patient was discharged after three weeks of hospitalization. The pathological result revealed the presence of moderately differentiated adenocarcinoma with two hepatic metastases and no involvement of the eighth paracolic resected lymph nodes. Medical imaging suggested $cT_3\ N_{1b}\ M_{1a}$ stage tumor, after the pathohistological examination the tumor stage was $pT_3\ N_{1c}\ M_{1a}$. Chemotherapy was prescribed which included oxaliplatin 85 mg/m^2, leucovorin 500 mg/m^2 and 5-fluorouracil 400 mg/m^2 with imaging follow-up in six months from now.

Discussions

The presence of SIT in a CRC patient could represent a challenge for the surgeon when choosing the laparoscopic approach as a treatment option. Some authors report the advantage of a left-handed surgeon, but a right-handed specialist can also perform the procedure by changing his position [4, 5, 6, 7]. Young Wan Kim et al. reported two cases of SIT. They emphasized the fact that this malformation could predispose to cancer [8]. In the literature some authors reported the occurrence of synchronous and metachronous gastrointestinal malignancies in patients with situs abnormalities (SIT or SA) suggesting the possibility of a connection between unidentified genes responsible for the arrangement of the left-right axis and cancer susceptibility [9]. However, it seems that the incidence of gastrointestinal malignancies in patients with situs abnormalities does not differ significantly from that of malignancies in patients with situs solitus [10].

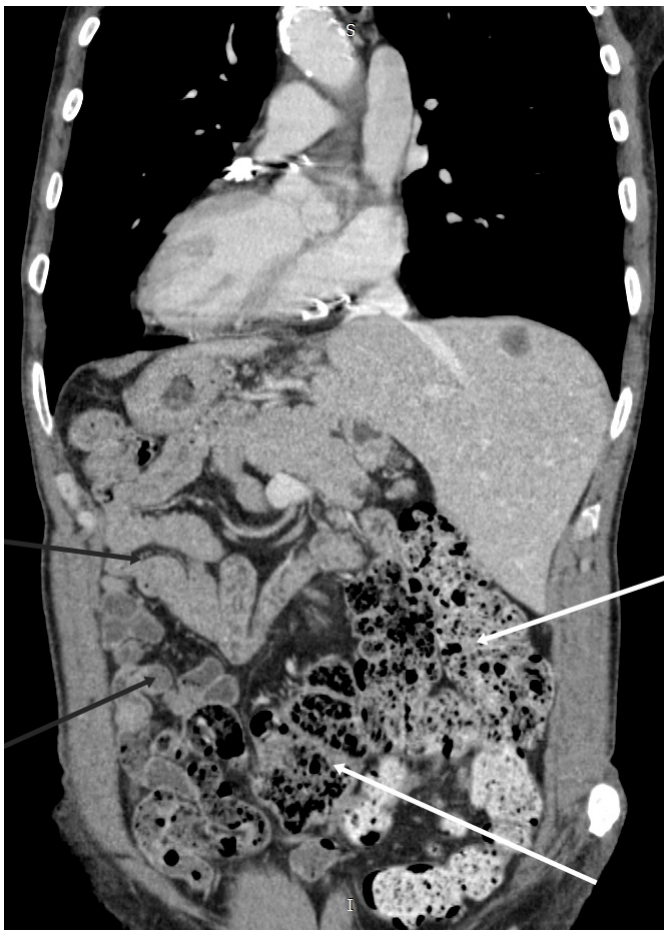

Fig. 1. CT scan image - contrast enhanced MPR coronal view, in portal venous phase, soft tissue window: image depicts dextrocardia, liver with hypovascular metastasis on the left side , large bowel on the left of the midline(white arrows), small bowel on the right of the midline(black arrows)

The anatomical findings represented by bilateral bilobed lungs and polysplenia could suggest a type of SA with double left-sidedness. However, just the right bronchus was hyparterial (bronchial course below the pulmonary artery), whereas the left bronchus was eparterial which strongly suggest the presence of situs inversus. Another anatomical feature was the shape of the atrial appendages, consistent with the anomaly of situs inversus. Polysplenia is also associated with SIT [11]. In the abdomen, the liver was on the left side and on the right side multiple spleens were observed. In most reported cases of polysplenia the agenesis of the body and tail of the pancreas were present. However, in our patient, the pancreas was normally developed, having all three segments [12, 13]. We observed interrupted inferior vena cava with the absence of the hepatic segment and azygos vein continuation. There are case reports of associated interruption of inferior vena cava and SIT, but this is an uncommon condition [14, 15]. The portal vein had a preduodenal course. There are reported associations among SIT or left isomerism and preduodenal portal vein which sometimes can lead to duodenal obstruction, especially in neonates [16, 17]. Another uncommon finding in our patient was the aspect of the small and large intestine. The stomach and the duodenum revealed a complete transposition, but the jejunum and the ileum were almost entirely located on the right side of the midline (Figure 1). As a mirror image of the stomach and the duodenum with retroperitoneal fixation of D2, D3 and D4, the jejunum should have been situated on the right side and the ileum on the left side. This was another particular anatomical aspect in our patient, since the ileocecal junction was placed in the front side of the midline, with the rest of the colon on the left side of the abdomen (Figure 3). This finding corresponds to a midgut nonrotation (mesenterium comune). However, in this case, the duodenum was retroperitoneal, with the presence of the ligament of Treitz. The superior mesenteric vein was placed to the right side of the superior mesenteric artery which represents an abnormal position for SIT [18]. This finding is consistent with an inverted rotation of the foregut, whereas the midgut was

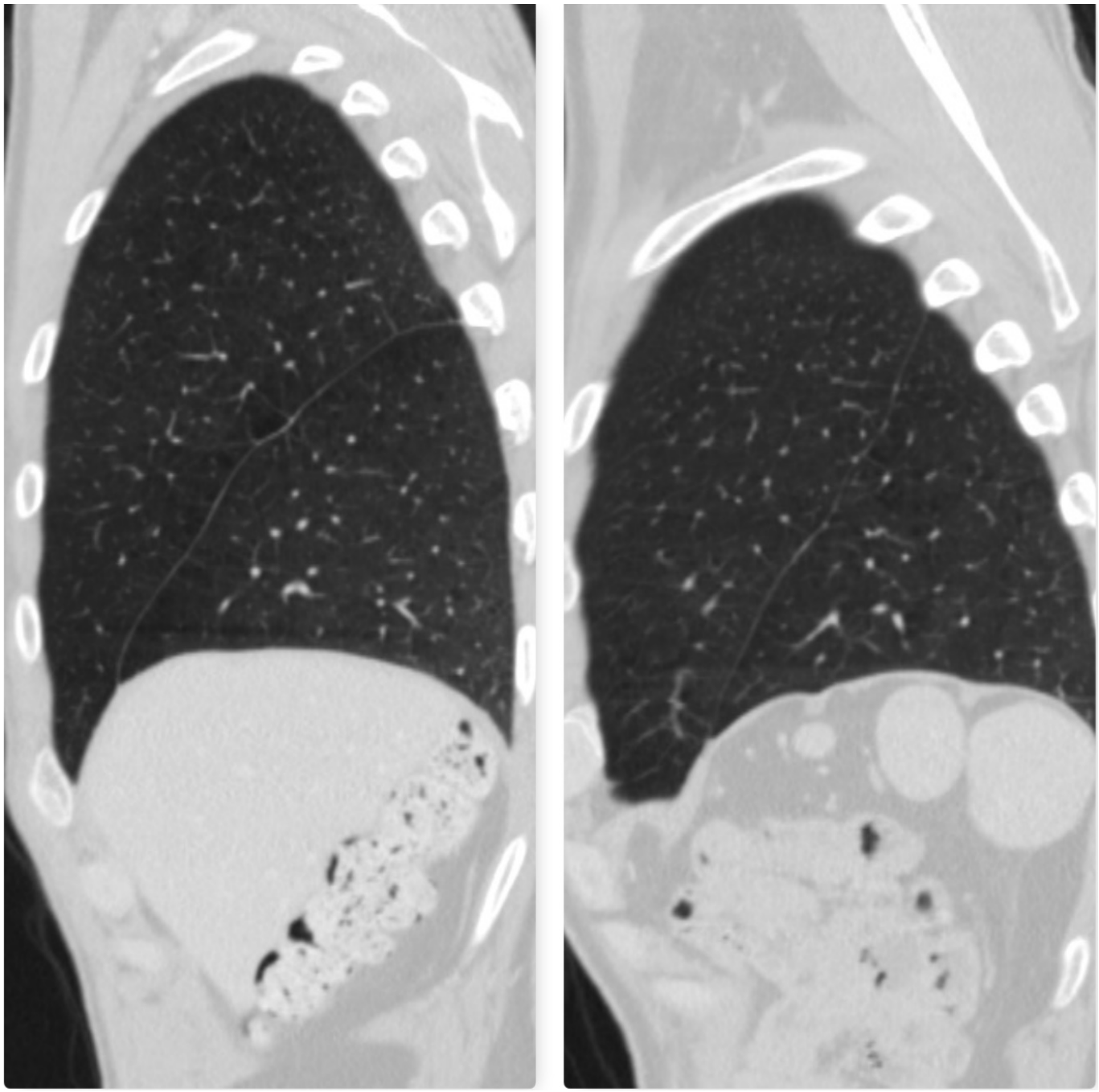

Fig.2. CT scan image – contrast enhanced MPR, sagital view, lung window: on the left, the oblique fissure of the left lung, no horizontal fissure, with liver and colon visualisation below diaphragm; on the right, the oblique fissure of the right lung, with multiple spleens and jejunum below diaphragm

nonrotated. Another case report of ascending colon cancer in a patient with intestinal nonrotation (mesenterium comune) emphasizes the importance of identification of malformations prior to surgery [19]. The presence of distant metastases without involved regional lymphnodes was also an unusual finding. However, tumoral cells were identified, microscopically, in the pericolic fat tissue. The presence of skip metastases involving intermediate colic lymph nodes with normal appearance could be considered. Lymph nodes with homogeneous structure and a short axis diameter up to 3 mm can contain malignant cells [20]. Brillantino et al., Nakayama et al. and Donner et al. reported cases of colon adenocarcinoma in T_3 stage, T_{4b} stage respectively, with negative regional lymph nodes in patients with intestinal malrotation [19, 21, 22]. Another 13 case reports of patients with intestinal malrotation and colon cancer revealed that the nonrotation type was the most common finding [21].

Conclusions

Situs abnormalities do not represent premalignant conditions, the incidence of malignancies in these patients being almost the same as in patients with situs solitus. Preoperative imaging in patients with CRC is necessary for a proper surgical management. The identification of situs abnormality is essential for the right surgical approach,

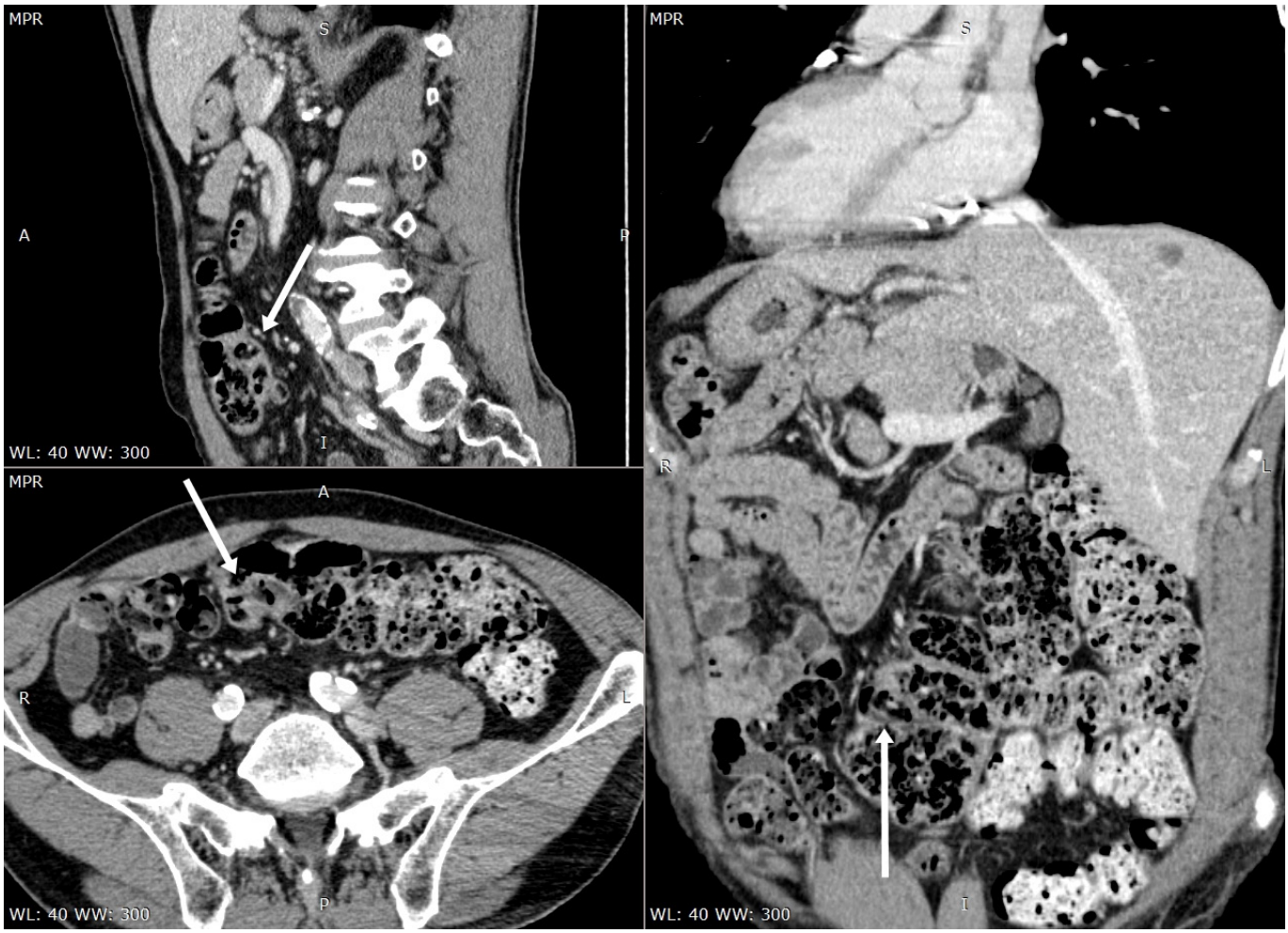

Fig.3. CT scan image – contrast enhanced MPR portal venous phase; white arrows indicate ileocecal junction(upper image sagital view, lower image axial view, right image coronal view)

especially where the laparoscopic technique is considered. Colorectal cancer metastasizing patterns in patients with intestinal malrotation may show unusual findings, further research being necessary. Furthermore, the possibility of metachronous or synchronous tumor incidence should not be overlooked.

Conflict of interest

None to declare.

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CASE REPORT

Late Onset Tay-Sachs Disease in a Non-Jewish Patient: Case Report

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Tay-Sachs disease (TSD) is a rare, inherited, autosomal rececessive lysosomal storage disease. The late-onset form is an uncommon condition among non-Jewish population.

We present the case of a 32 years old male patient without Jewish origins, in whom the disease began in adolescence and was initially diagnosed with spinal muscular atrophy. He developed progressively protean neurological symptomatology, including tetraparesis, cerebellar and extrapyramidal syndromes. The diagnosis was based on the cerebral MRI, showing severe cerebellar atrophy and the determination of the Hexosaminidase A activity, revealing low level.

In patients showing signs of lower motor neuron involvement, cerebellar and pyramidal signs and marked cerebellar atrophy the late-onset TSD should be suspected, and the first step in establishing the diagnosis should be to determine the serum activity of Hexosaminidase A.

Keywords: Tay-Sachs disease, autosomal rececessive , hexosaminidase A

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Introduction

Tay-Sachs disease (TSD) is the most frequent form of the GM2-gangliosidoses, a heterogenous group of lysosomal storage diseases that are inherited in an autosomal rececessive manner. Hexosaminidase (Hex) A is a lysosomal enzyme, which degrades the ganglioside GM2, and is composed of an alfa subunit encoded by the HEXA gene and a beta subunit encoded by the HEXB gene. In TSD, a mutation in the HEXA gene, causes a deficiency of HexA, resulting in an accumulation of GM2 gangliosides in the nervous system. (1)

TSD has two clinical forms of evolution. The most common form is the infantile or acute TSD, with severe reduction of HexA activity (less than 0.5%), characterized by a rapid evolution, leading to death between the ages of 3 and 5 years. The second form, late-onset TSD is associated with a higher HexA activity of 2-5% of normal levels and has a more prolonged clinical course. It is, in turn, subdivided into juvenile or sub-acute form which usually leads to a vegetative state before the age of 15 and adult or chronic form, with onset in late childhood, in adolescence, or even later. Residual enzyme activity of over 10% does not cause clinical manifestations. (2, 3, 4, 5)

TSD has an increased prevalence in the Ashkenazi Jewish population, even if the introduction of carrier screening programs and prenatal diagnosis has led to a decrease in the incidence of TDS with approximately 90% in the Jewish population. (1)

Late-onset TSD is considered to be a rare condition among people without Ashkenazi Jewish ancestry. (2, 6)

We present the case of a patient without Jewish origins, in whom the disease began in adolescence and was initially diagnosed with spinal muscular atrophy.

Case report:

A 32 years old male patient, with negative medical and family history, without consanguinity, developed after the age of 17 an inconstant clumsiness and mild tremor in the upper extremities. At the age of 19 he developed a predominant proximal muscle weakness in the lower extremities, with slowly progressive aggravation, later he developed muscle atrophyes and fasciculation in the thigh and shoulder girdle muscles. At the age of 25 he presented swallowig difficulties, dysartria, coordination and equilibrium problems. He was diagnosed initially with spinal muscular atrophy.

At the age of 32 he was addressed to our department with the suspicion of amyotrophic lateral sclerosis. The neurological examination at the admission revealed bilateral horizontal nystagmus, without gaze abnormalities, diminished pharyngeal reflexes, dysphagia for solid foods, paraparetic and ataxic gait, tetraparesis with grade 4 motor deficit in the upper extremities, grade 3 in the proximal muscle groups and grade 4 in the distal muscle groups of the lower limbs, exaggerated deep tendon reflexes in the upper extremities, diminished deep tendon reflexes in the lower extremities, bilateral plantar clonus, flexor plantar response, right sided Rossner sign, positive palmomental reflex, cogwheel sign in the upper extremities, postural tremor of the extremities, truncal ataxia, bilateral dysmetria, generalysed muscle atrophies, fasciculations in the thigh and shoulder girdle muscles, dysartria, normal cognition.

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The laboratory examinations (complete blood count, biochemistry, Erithrocyte Sedimentation Rate, serum protein immunoelectrophoresis, autoimmune markers, VDRL, thyroid hormones) were in normal range.

The cerebral and spinal cord magnetic resonance imaging exam was normal, excepting a severe cerebellar atrophy. (Figure 1, 2)

The electrodiagnostic studies show normal motor and sensory nerve conduction parameters. The needle EMG examination shows chronic neurogenic changes in the lumbar and cervical myotomes. (Figure 3.) The electrophysiologic findings are consistent with chronic generalized disorder of the motor neurons, their axons, or both, in a context of a a slowly progressive degenerative disorder.

The psychological workup revealed normal cognition, MMSE 30 p, normal clock-drawing test result (10/10), without psychiatric symptoms.

The ophtalmological examination revealed normal conditions.

The HexA and HexB activities were measured, the HexA activity was low (0.02 nmol/spot, normal values: 0.6-2.4 nmol/spot).

The diagnosis of GM2 gangliosidosis, adult onset Tay-Sachs disease was established. The genetical analysis was not possible.

Discussions

Late-onset TSD is an extremely rare condition among non-Jewish people, its real incidence is unknown, but is considered to be somewhere around 1 case per 300,000

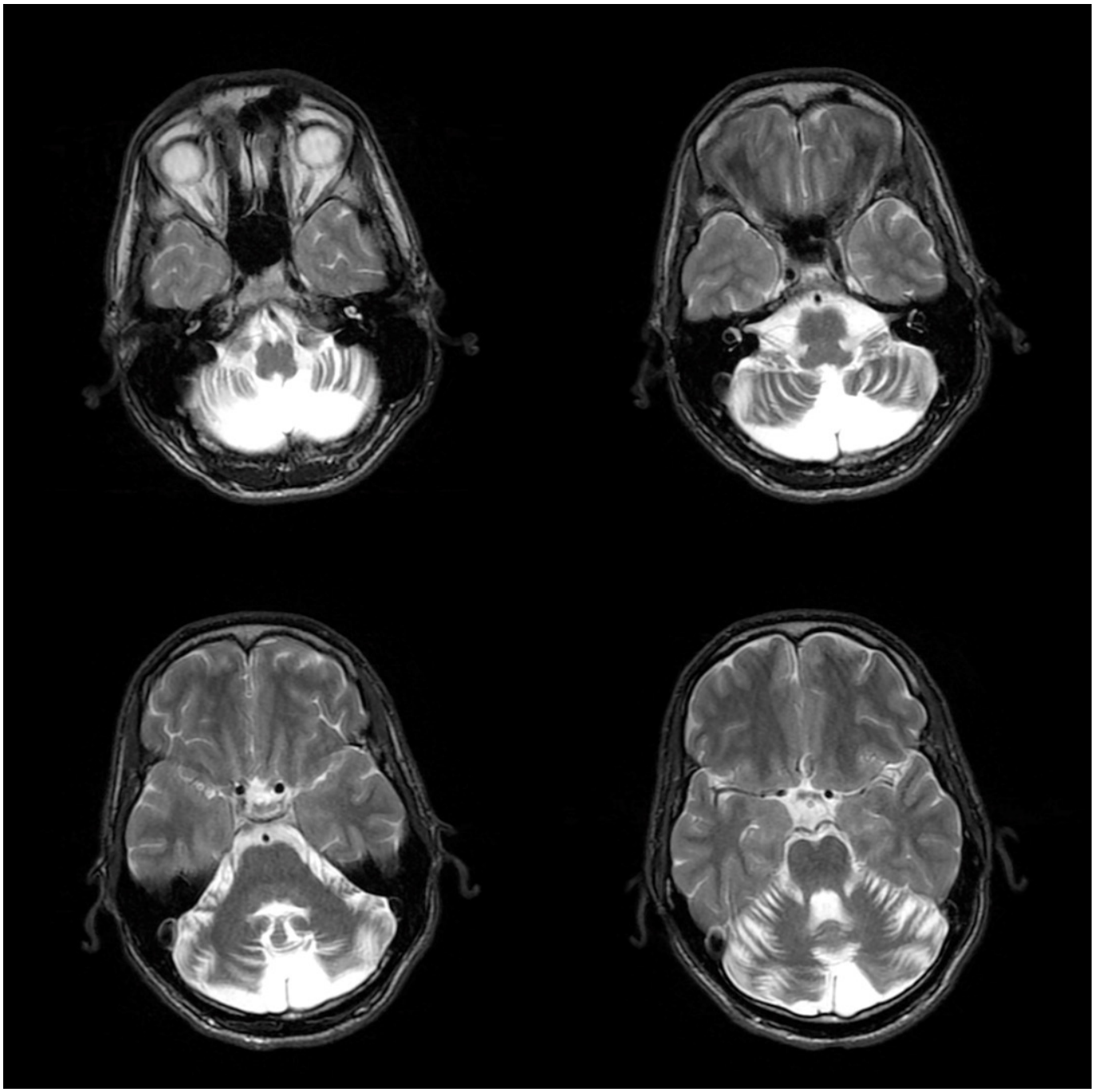

Fig. 1. Axial T2 weighted cerebral MRI revealing severe cerebellar atrophy

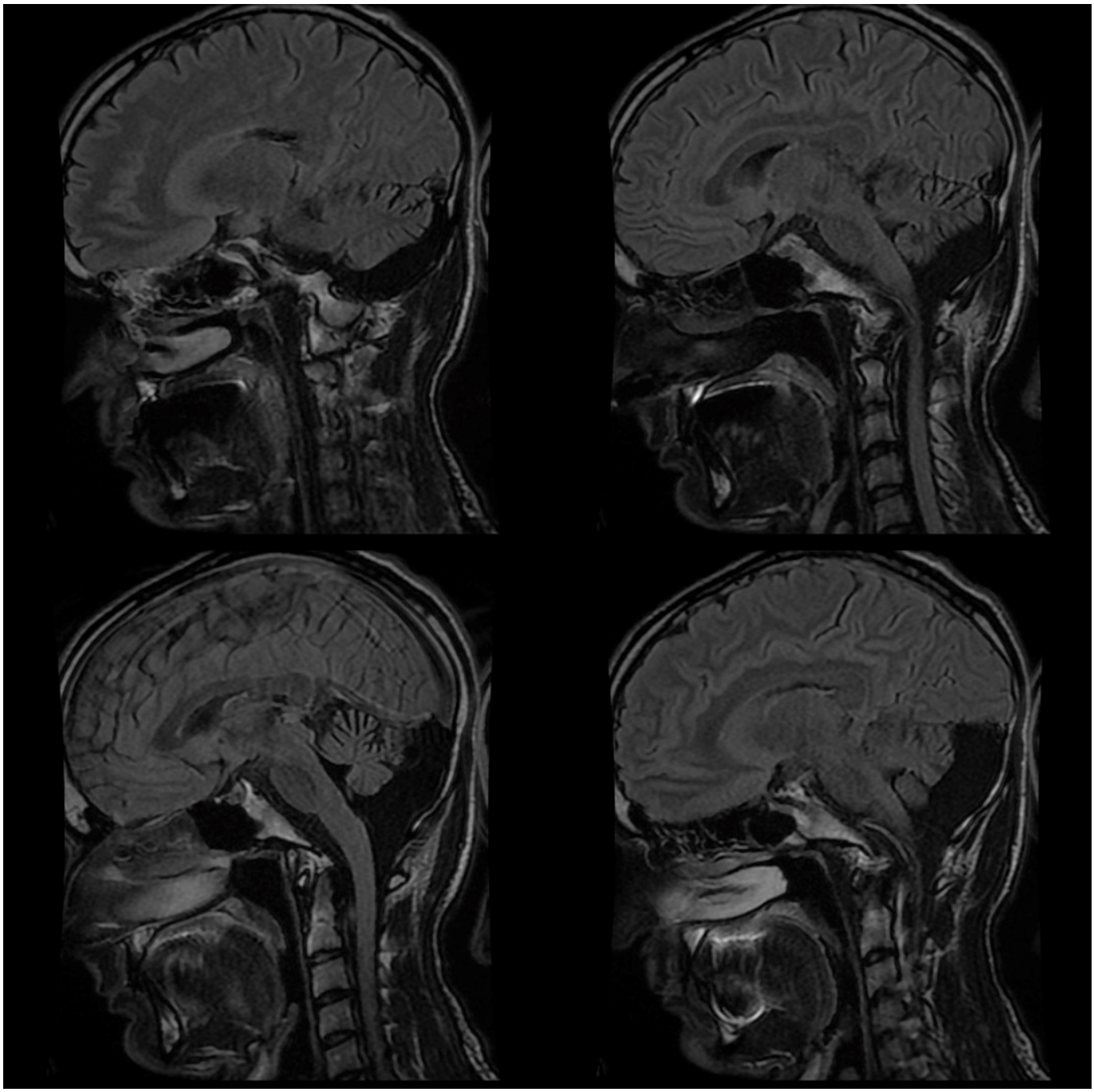

Fig. 2. Sagittal T2 FLAIR weighted cerebral MRI revealing severe cerebellar atrophy

people. In the Ashkenazi Jewish people its incidence is 1 case per 135,000 people. (2)

In patients with late-onset TSD, HexA activity is decreased between 2-5% of normal values, thus causing a late onset and a slower evolution in deterioration of motor, cerebellar and spinocerebellar functions.(3) In some patients, the disease begins in adolescence with cerebelar (dysarthria, ataxia, tremor, dysmetria) or motor signs (proximal motor weakness, atrophies, fasciculations) and in others the predominant symptoms at onset are those of a psychiatric nature (psychosis, recurrent depression, bipolar disorders). (2) In the case of our patient, psychiatric symptoms are completely absent, the disease being manifested at onset by cerebellar and motor signs.

A delay in the diagnosis of late-onset TSD of 15 years in our case, was also found by Neudorfer O et al. They performed a study on 21 patients with late-onset TSD and observed that 17 patients were correctly diagnosed late, about 8-10 years after the onset of the symptoms, the initial diagnosis being of muscular spinal atrophy, amyotrophic lateral sclerosis or spinocerebellar ataxia.(2) Our patient was initially diagnosed with spinal muscular atrophy.

Considering the predominance of the proximal motor deficit at the onset of the disease, besides the diagnosis of spinal muscular atrophy, GM2 gangliosidoses should also be considered in the case of our patient, and dosing of the HexA enzyme would have been an adequate step for establishing earlier the right diagnosis. (7-9)

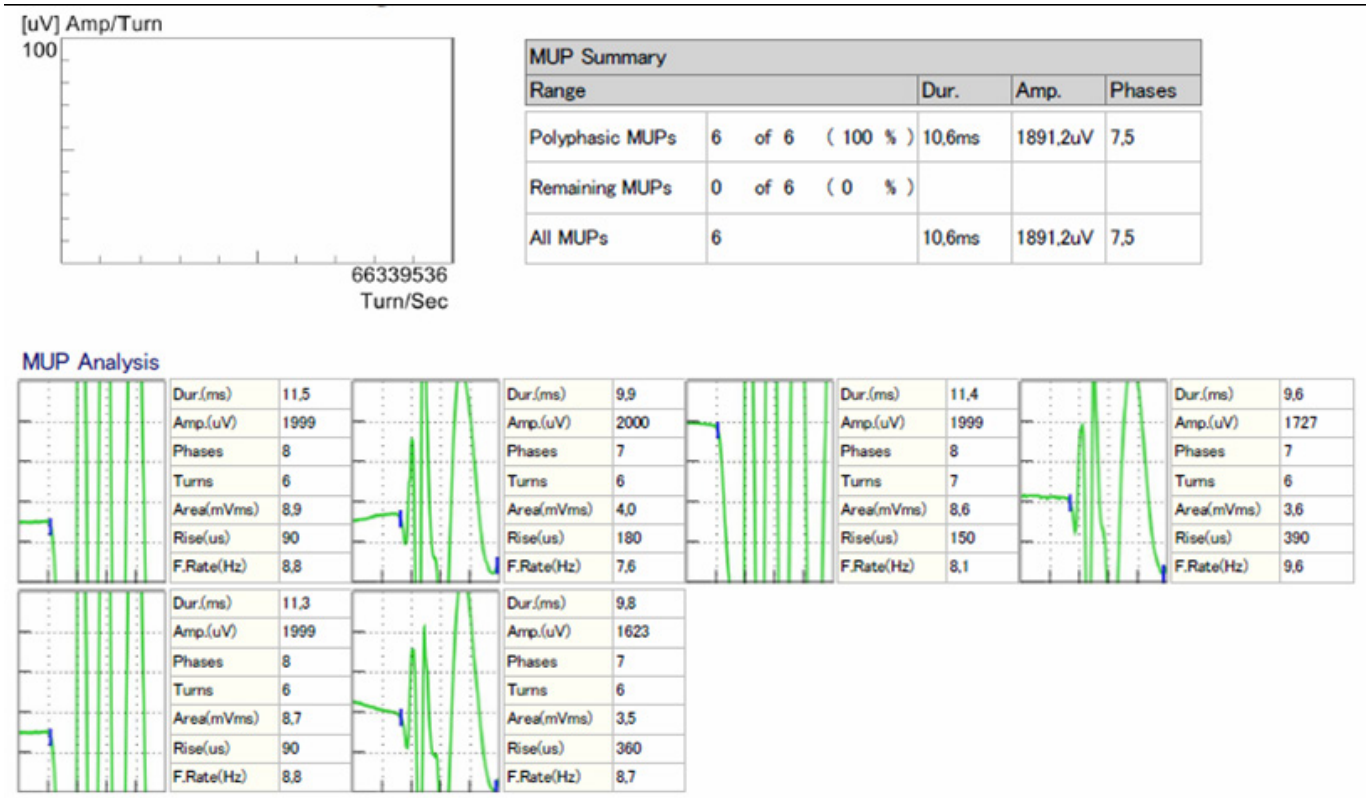

Fig. 3. EMG examination. Motor unit potentials with neurogenic changes in vastus lateralis muscle.

An important feature of the disease is marked cerebellar atrophy, especially of the vermis, which becomes evident in the second decade of life in brain imaging exams. There is a poor correlation between the degree of cerebellar atrophy and the patient's symptomatology. Despite severe cerebellar atrophy, our patient was independent.(1, 10, 11)

During the course of the disease, extrapyramidal signs may also occur, in some cases manifesting as progressive generalized dystonia, usually associated with cognitive decline. In our case extrapyramidal syndrome is manifested by cogwheel sign in the upper extremities, postural tremor of the extremities, but the cognitive functions are normal. (1)

Our case emphasize the emergence of protean clinical characteristics over many years and accentuate the need to consider late-onset TSD in the differential diagnosis of progressive muscular atrophy and early onset amyotrophic lateral sclerosis and even primary lateral sclerosis(8, 9, 12)

A study conducted by Gustavo H.B. Maegawa et al. had some promising results. It showed that pyrimethamine can be used as a potential pharmacological chaperone in late-onset TSD as it intensify the activity of the remaining HexA levels. (4) Another study, performed by Osher E et al demonstrated that this increase is transient and has no benefit on neurological and psychiatric symptoms.(3)

Even if there is still no effective treatment for late-onset TSD, early accurate diagnosis is particularly important because certain drugs, especially neuroleptics, can aggravate the symptoms and should be avoided in these patients. Also a late diagnosis may delay proper genetic counseling regarding disease progression and reproductive risks, which is especially important in Ashkenazi Jewish patients because the disease is transmitted in autosomal recessive fashion. (2, 13)

In patients showing signs of lower motor neuron involvement, cerebellar and pyramidal signs and marked cerebellar atrophy the late-onset TSD should be suspected, and the first step in establishing the diagnosis should be to determine the serum activity of HexA.

Acknowledgment

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Conflict of interest

None to declare.

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