doi: 10.4183/aeb.2015.46

KETOCONAZOLE TREATMENT IN CUSHING'S SYNDROME – RESULTS OF A TERTIARY REFERRAL CENTER IN ROMANIA

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Abstract

Introduction. First election treatment in Cushing's syndrome is the surgical therapy (pituitary or adrenal). Pharmacotherapy is used: before surgery, when the surgery was ineffective, in association with radiotherapy or in cases of refuse or contraindications for surgery.

Aim of the study. Testing the effectiveness and safety of Ketoconazole treatment in patients with Cushing's syndrome.

Methods. We studied 12 patients with Cushing's syndrome treated with Ketoconazole between 2010 and 2013. We followed cortisol levels before and during treatment, the doses of Ketoconazole and the time required for normalization of cortisol, "the escape syndrome" and the adverse effects.

Results. Eleven (91,66%) patients had ACTH – dependent Cushing's syndrome. The mean basal cortisol before initiation of the therapy was 404.4 ± 71 ng/ml. Two thirds (eight) patients presented a normalization of serum cortisol levels with 300-800 mg Ketoconazole/day, during a mean of 8.5 weeks. Only one patient presented an "escape syndrome" and one presented adrenal insufficiency. None of the patients showed significant side effects under the treatment.

Conclusions. Ketoconazole therapy is well tolerated and is effective in most patients with Cushing's syndrome even in long term use. The resistance and the escape from the effect of the treatment is possible, but rare, patients requiring close monitoring during therapy.

Key words: Cushing's syndrome, Ketoconazole, adrenalectomy.

INTRODUCTION

Cushing's syndrome (CS) is a severe condition due to endogenous or exogenous cortisol excess. The most common cause of endogenous CS is Cushing's disease (CD) due to an adrenocorticotropic hormone (ACTH) secreting pituitary tumor. Other causes of endogenous Cushing's syndrome are: adrenal adenomas and adenocarcinomas, bilateral adrenal micro or macronodular hyperplasia and ectopic ACTH syndrome (EAS) (1).

Due to increased morbidity and mortality rates, timely and definitive treatment is mandatory. Tumor-directed surgery is the first-line treatment for virtually all patients with Cushing's syndrome whether it is caused by a pituitary adenoma, an ectopic ACTH secreting tumor, adrenal adenoma, adenocarcinoma or nodular hyperplasia. For Cushing's disease (CD) the second line therapy comprises anti-tumoral radiotherapy. In patients with ACTH-dependent Cushing's syndrome where the surgery and radiotherapy are not curative and in patients with bilateral nodular hyperplasia, laparoscopic bilateral adrenalectomy offers a safe and effective mean of curing the hypercortisolemia, but the consequence is hypoadrenalism that must be treated by replacement therapy for the rest of the life.

Medical therapy to control hypercortisolism uses a number of drugs acting: at the level of the secreting pituitary tumor (Cabergoline, Pasireotide), at the level of the adrenal steroidogenesis (Mitotane, Ketoconazole, Metyrapone, Etomidate) or at the level of cortisol receptors (Mifepristone). Other drugs have been tested over time or are currently in trials but their efficacy was low or has not yet been proved (aminoglutethimide, retinoic acid, peroxisome proliferator-activated receptor γ "PPAR-γ" ligands, temozolomide and tyrosine kinase inhibitors, etc.). All the drugs in use now have advantages, but also have more or less severe side effects (1-7) that must be carefully balanced when choosing a medical treatment (Table 1). Another problem is that not all the drugs are available in every country, so the treating doctors have to deal with those available.

Pharmacotherapy is used in several circumstances as shown below:

in severe cases of Cushing's syndrome, in preparation of surgery, in order to reduce peri-operative morbidity and mortality;

when the surgery was ineffective, while

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considering further surgery such as pituitary reintervention or bilateral adrenalectomy;

after pituitary radiotherapy, waiting for its effects, which may take several years;

in patients with ACTH dependent Cushing's syndrome when the source of ACTH is uncertain, in consequence the surgical treatment does not have a precise target. These patients will be reevaluated after 6-12 months of medical treatment;

in patients with inoperative metastatic adrenocortical carcinoma or inoperative ectopic tumor secreting ACTH, in order to reduce morbidity due to cortisol excess;

in the patients that refuse surgery and radiotherapy;

if the psychiatric complications of hypercortisolism are an immediate threat to the patient's safety (4, 6).

As shown in Table 1, a number of drugs have been advocated for the medical management of Cushing's syndrome, but only few have gained widespread acceptance. To date, the most reliably effective agents are Metyrapone and Ketoconazole as monotherapy or in combination (9, 10). In the last three decades, Ketoconazole, an antifungal agent, has proved to be an effective and safe inhibitor of steroidogenesis; therefore it is used off-label in the treatment of Cushing's syndrome. Ketoconazole inhibits 11 beta-hydroxylase and 17, 20-lyase in the steroid synthetic pathway and it decreases the production of cortisol and androgens. The recommended initial dose is 200 mg twice a day, which, if tolerated, can be increased to 400 mg three times a day as a maximal dose. It takes several weeks to achieve its full effect on cortisol levels. (1, 5-10). Long-term treatment with Ketoconazole has been reported to control hypercortisolism in 51.5% and 64.7% respectively of

Table 1. Drugs used in the treatment of Cushing's syndrome and their availability in Romania (adapted from 7, 24, 25)

Name	Action	Doses	Comments	Adverse effects	Indication
CABERGOLINE (available in Ro)	Dopamine agonist	2-8 mg/ week orally	Low efficacy	Nausea, dizziness, cardiac valve dysfunction?	Cushing's disease
PASIREOTIDE (recently available in Ro)	Somatostatin analogue with high affinity for receptor subtypes 1,2,3 and 5	600-1200 µg/day s.c.	Partial efficacy and association with hyperglycemia in more than 50% of patients	Nausea, abdominal pain, diarrhea, Hyperglycemia	Cushing's disease
METYRAPONE (not available in Ro)	Inhibitor of cortisol synthesis by blocking 11 hydroxylase	750-8000 mg/day orally	Rapid onset of action (4-6 days)	Hypoadrenalism, (nausea, anorexia, abdominal pain) Hirsutism and acne in women	Cushing's syndrome
KETOCONAZOLE Antifungal agent (no more available in Ro)	Inhibitor of steroid hormones synthesis by blocking 11beta-hydroxylase and 17,20-lyase	400- 1200 mg/day orally	Slow onset of action (4-8 weeks) Reduces also the level of total cholesterol and LDL	Rarely hypoadrenalism Antiandrogenic effect resulting in gynecomastia and reduced libido in men. Liver enzyme dysfunction in 10% of cases Skin rashes	Cushing's syndrome
MITOTANE Cytotoxic agent (available in Ro as oncologic treatment)	Reduces cortisol synthesis by blocking cholesterol side chain cleavage and 11hydroxylase	0.5- 4g/day orally	Slow onset of action, but sustained action after discontinuation	Nausea, anorexia, diarrhea Hypoadrenalism Neurological symptoms: abnormal gait, dizziness, vertigo, confusion, slow language expression. Abnormal liver enzymes, hypercholesterolemia, hypouricemia, prolonged bleeding time, skin rash, gynecomastia	Adrenocortical carcinoma but also other forms of Cushing syndrome resistant to other treatments
ETOMIDATE Parenteral anesthetic (available in Ro)	Inhibits cortisol synthesis	2.5 mg/hour i.v.	Rapid onset of action in i.v. administration	Short duration of treatment due to i.v. administration	Acutely sick patients that cannot be treated orally.
MIFEPRISTONE (not available in Ro)	Potent antagonist of the glucocorticoid and progesterone receptors	300-200 mg/day orally	There is no marker of disease activity (cortisol can't be used)	Amenorrhea, hypokalemia, increased blood pressure, edema, or alkalosis	Ectopic ACTH syndrome or other forms resistant to other treatments

patients with CD (8, 10). The commonest adverse effects reported include: elevated aminotransferases (ASAT, ALAT) and gamma glutamyl transferase (GGT), gastro-intestinal disturbances and rash. Acute liver failure is very rare. These adverse effects usually appear in the first week of treatment or after increasing the dose. Due to its anti-androgenic effects, men may report deterioration of libido, erectile dysfunction or gynecomastia (9), but these symptoms are already present in CS. For women the anti-androgenic effect is beneficial considering the hyperandrogenism present in many forms of CS. Ketoconazole has also the benefit of reducing the total cholesterol and low-density lipoprotein (LDL) cholesterol, which are increased in CS patients (5).

Our study is retrospective and analytic, testing the efficacy and safety of Ketoconazole in the patients with Cushing's syndrome treated in a tertiary reference center in Romania. The study was approved by the medical ethical commission of the hospital.

The study has been completed in May 2013, several months before the decision of European Medicines Agency (EMA) to suspend Ketoconazole from the market in his main indication: the fungal infections, due to high risk of liver function impairment. EMA also stipulated that "The European Medicines Agency is aware that ketoconazole is used off-label for treating patients with Cushing's syndrome. In order to ensure that these patients will not be left without treatment, national competent authorities may make these medicines available under controlled conditions" (11). In the light of this fact, our study comes to bring arguments that Ketoconazole is an effective drug as steroid inhibitor in CS, and it is safe enough to be maintained for this particular indication.

PATIENTS AND METHODS

We retrospectively studied 12 patients with Cushing's syndrome treated with Ketoconazole between January 2010 and May 2013. Diagnosis of Cushing's Syndrome was established according to international guidelines (12). We assessed: morning serum cortisol levels before and during treatment, the doses of Ketoconazole and the time required for normalization of serum cortisol, "the escape syndrome" and the side effects: gastro-intestinal disturbances, increase of the hepatic enzymes (one month after the start of Ketoconazole treatment or the increase of dose), hypoadrenalism, gonadal insufficiency, gynecomastia and rash. The evolution of ACTH, testosterone, lipids, glucose tolerance, blood pressure and weight were also recorded. The patients were considered controlled if the morning cortisol was within the normal ranges. Improvement in hypertension was defined as a decrease of at least 10 mm Hg of systolic and/or diastolic BP in patients with hypertension. Improvement in lipid profile was defined as a decrease of at least 10 mg/dL of cholesterol and/or triglycerides. Improvement of glycemic control was defined as a decrease of insulin dose > 10%, or a decrease in the number of antidiabetic drugs, or an improvement of glycosylated hemoglobin (HbA1c) >0.5%. Improvement of weight was defined as a decrease in body index mass > 2.

In the analysis of qualitative variables the χ^2 test was used. Comparison of means was evaluated with Student's t-test. All statistical tests were two-tailed and $p<0.05$ was considered significant.

RESULTS

Thirty-three patients with Cushing's syndrome of different etiologies were identified in our unit's evidence between January 2010 and May 2013. The mean age was 42.33 years (4-72 years), with a preponderance of female cases (F:M = 26:7). 19 of these patients (57,57%) were cured by surgery, 12 patients were treated with Ketoconazole, and two (with nodular adrenal hyperplasia) are not yet treated. This shows that one third of CS patients needed medical treatment at one point of their evolution.

In the group of 12 patients treated with Ketoconazole the mean age was 35,9 years (4-63 years) and there was a slight preponderance of female cases (F:M = 7:5). Eleven of the patients were diagnosed with ACTH – dependent Cushing's syndrome (7 cases with CD, 3 cases with CD without visible lesion on magnetic resonance imaging (MRI), one case of ectopic ACTH syndrome) and one case of nodular adrenal hyperplasia (Fig. 1).

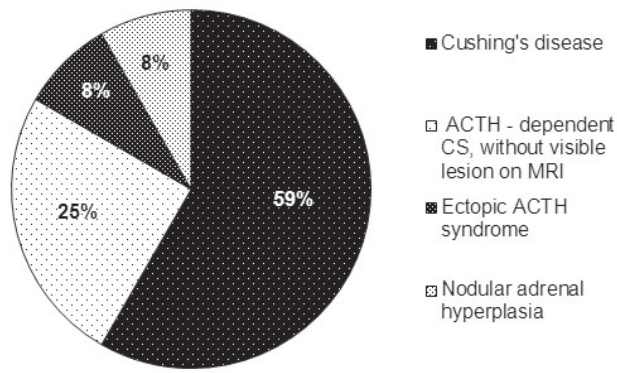

Figure 1. The etiology of CS in the 12 patients with CS treated with Ketoconazole.

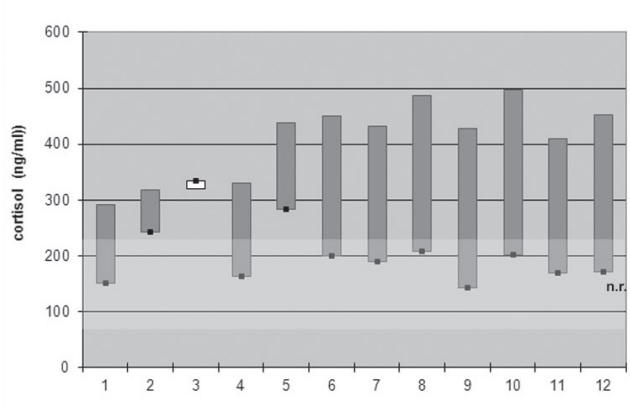

Figure 2. Cortisol levels: before initiation and the lowest level reached with Ketoconazole treatment.

The reasons for initiating the ketoconazole treatment were as follows: in preparation of surgery (4 patients); unsuccessful pituitary surgery (one patient); two patients uncured after pituitary surgery and radiotherapy; lack of image of adenoma on pituitary MRI (3 patients); ectopic ACTH syndrome (one patient), waiting for efficacy of conventional radiotherapy (one patient).

The mean basal cortisol before initiation of Ketoconazole therapy was 404.4 ± 71 ng/mL with range 291.2 - 497 ng/mL, (n.r. 70 - 225 ng/mL). The mean follow-up was 15.5 months; range 1 - 75 months. Cortisol levels were significantly lower under treatment: 212.73 ± 71.01 *versus* 404.4 ± 71 ng/mL ($p < 0.0001$).

Table 2. Individual values of the 12 patients treated with Ketoconazole. CD= Cushing disease, AMiH= Adrenal micronodular hyperplasia, EAS= Ectopic ACTH secretion, MRI= magnetic resonance imaging, ACTH= adreno corticotrophic hormone, UFC= urinary free cortisol, NI= no tumoral pituitary image in IRM

Cr . Nr.	Sex	Diagnostic	MRI	The reason for initiating Ketoconazole	Bazal cortisol (nr 70-225ng/ml)	Cortisol level under treatment (ng/ml)	Eficient dose of Ketoconazole (mg/day)	Time to normalisation (months)	Time to escape (months)	Duration of treatment (months)	basal ACTH (pg/ml)	tr ACTH (pg/ml)	basal UFC (nr 5-19 µg/24h)	tr UFC (nr 50-190 ug/24h)	basal Testosterone (nr=2-10 ng/ml in men, **0.2-1 ng/ml in women)	tr Testosterone	at the end of the study
1	M	CD	NI	no tumoral image on MRI	**291.2**	**152**	**600**	2		9	82.1		300		9.25	7.1	under treatment
2	M	CD	NI	after radiotherapy	**317.4**	**242.8**	**800**	**NC**		26	50.3	42.5			1.26	0.5	pasireotide
3	F	CD	MI	after surgery	**319.9**	**334.2**	**400**	**NC**		18	95	88.6			0.94**	0.5	pasireotide
4	F	AMiH	NI	before surgery	**329.7**	**163.2**	**600**	2.5		7	<5	33.6			2.93**	1.04	left adrenalectomy
5	M	CD	MI	before surgery	**438.2**	**283**	**800**	**NC**		1	97.6		1167		0.95	0.6	pituitary surgery
6	M	CD	MA	after surgery and radiotherapy	**451**	**201**	**800**	1	1	4	438	372	506		1.31	0.9	bilateral adrenalectomy
7	M	CD	MI	before surgery	**432.9**	**191**	**400**	0.5		15	50.2				5.09	1.9	pituitary surgery
8	F	CD	MI	before surgery	**486.4**	**207.9**	**400**	2		4	48.9	86.4			3.17**	0.8	pituitary surgery
9	F	CD	NI	no tumoral image on MRI	**428.41**	**143.4**	**800**	3		5	41.1	34.7			6.1**	0.2	under treatment
10	F	CD	MI	after surgery and radiotherapy	**497**	**333.9**	**600**	**NC**		75	53.8	33		275.7			pasireotide
11	F	CD ROHADNET	NI	no tumoral image on MRI	**408.9**	**170**	**300**	4		4	66.7	28.9	205.4	107.88			deceased*
12	F	EAS	NI	no tumoral image on MRI/CT	**452.8**	**130.4**	**600**	0.5		18	144	54.4			6.58**	0.1	deceased

* see ref. 13

Eight patients showed normalization of cortisol with 300-800 mg Ketoconazole/day, in a mean of 8.5 weeks (2-16 weeks). The control rate in the group of treated patients was 66%. Only one patient (with corticotroph macroadenoma) presented an "escape syndrome", with 800 mg Ketoconazole/day, one month after cortisol normalization and the control was not restored despite increasing the dose at 1000 mg/day, in consequence bilateral adrenalectomy was performed. Finally, from the group of controlled patients, two were referred to pituitary surgery, two were referred to adrenal surgery, two patients died during the treatment (one, with ROHHADNET syndrome, died of cardiorespiratory arrests (13); the other, with EAS, died by sepsis), and the other two, without a visible lesion on MRI, were still controlled under treatment with Ketoconazole at the end of the study.

In two patients the treatment was partially efficient: a decrease of 23% respectively 35% in cortisol level was obtained, without normalization; but one of them was treated with an inadequate dose (400 mg/day) and another only for one month. Despite these inaccuracies, the level of cortisol lowered close to the upper normal limit in both patients. In other two cases the normalization of cortisol levels was not achieved despite the use of doses of 600 mg and, respectively 800 mg/day for a period of 75 and, respectively 26 months. The patient treated for 75 months presented a partial control during the first 24 months. From this group of 4 patients with partial response, one patient was referred to surgery and the other three were treated with Pasireotide (Table 2). The evolution of cortisol levels of each patient treated with Ketoconazole is represented in Fig. 2.

The mean ACTH levels were 97.38 ± 113.10 pg/mL before treatment and 86.01 ± 109.62 pg/mL, with no significant difference between the two moments (p= 0.82). In only one patient with CD (patient 8) an increase in ACTH was recorded, in the others, there was a tendency to decrease. Interestingly, an important decrease was noted in the patient 12 with EAS (Table 2).

Testosterone levels were slightly decreased by the Ketoconazole treatment: 1.36 ± 2.07 ng/mL *versus* 3.57 ± 3.6 (p= 0.15). From the five male patients, three had low testosterone levels as result of their previous pituitary treatments (surgery and/or radiotherapy). The other two males, with normal values before Ketoconazole treatment, both showed a decrease in testosterone levels, but only in one the levels dropped under the normal range. In female patients testosterone levels decreased from 3.94 ± 2.35 ng/mL to 0.52 ± 0.39 ng/mL (p= 0.012), with normalization in all patients (Table 2).

Urinary free cortisol (UFC) was not currently available in our service, but in the few cases when it was available, it was concordant with the serum cortisol.

We compared the group of patients in whom Ketoconazole treatment was effective with the group of patients partially or uncontrolled with Ketoconazole and we did not find any statistically significant difference between the two groups of patients in terms of age (p= 0.36), sex (p= 0.67), cortisol level at initiation (p= 0.37), mean follow-up (p= 0.25) and Ketoconazole doses (p= 0.46). Initial cortisol level was not a predictive factor of treatment efficacy (Table 3).

The tolerability of treatment was good, with no interruption for adverse effects. One patient (with EAS) developed hypoadrenalism, one week after the initiation of treatment with 800mg/day, but normalization of cortisol levels was obtained by lowering the doses to 400 mg/day. In the further evolution the doses had to be increased progressively to 800 mg/day in order to maintain the control.

None of the patients showed increase of the hepatic enzymes under the treatment. Two patients with increased basal levels (patients 5 and 6) showed decreased levels during Ketoconazole treatment (Table 4). None of the male patients had increase of the previous gynecomastia or complaints related to decreased libido or sexual dysfunction. Cholesterol levels were improved in 7 patients, increased in two and stationary in three, with no significant difference between the mean values (p= 0.56). Triglyceride levels were decreased in five patients, increased in five and unchanged in two, with no significant difference between means (p= 0.71). Eight patients had hypertension, and six were improved by the Ketoconazole treatment. The only patient with diabetes treated with insulin (patient 6) showed a significant improvement by lowering the doses with 10 UI; two other patients with low glucose tolerance were normalized under Ketoconazole treatment. Ten of the patients were overweight or obese; during the treatment only five of them showed a significant loss of weight (BMI-2), with no significant difference between means (p= 0.39) (Table 4).

Table 3. Comparison between the two groups of patients (controlled and uncontrolled with Ketoconazole treatment)

	Controlled	Uncontrolled	p
No. of patients	8	4	
Mean age (years)	32.25	43.25	0.36
Sex ratio (F:M)	5:3	2:2	0.67
Initial cortisol (ng/mL)	410.16	393.12	0.37
Follow-up (months)	7.28	30	0.25
Ketoconazole doses	562.5	650	0.46

Table 4. Individual values of liver enzymes, cholesterol, triglycerides, blood pressure, glucose tolerance, psychiatric disorders, osteoporosis and body index mass. ASAT= Aspartate Aminotransferase, ALAT= Alanine Aminotransferse, GGT= Gamma-glutamyl Transpeptidase, BP= Blood pressure, HBP= High Blood pressure, DM= Diabetes Mellitus, IGT= Impaired glucose tolerance, BMI= Body index mas, *= Patients treated with specific drugs (for hypertension and dyslipidemia), tr = values when the patients were controlled with Ketoconazole treatment, or under maximal doses of treatment

Cr. Nr.	basal ASAT (nr= <34 UI/L)	tr ASAT (UI/L)	basal ALAT (nr= < 34 UI/L)	tr ASAT (UI/L)	basal GGT (nr=< 60 UI/L)	tr GGT(UI/L)	basal Cholesterol (nr= 140-200 mg/dl)	tr Cholesterol (ng/ml)	basal Triglycerides (nr= 50-150 mg/dl)	tr Triglycerides (mg/dl)	basal BP (mmHg) (*=HBP treatment)	tr BP (mmHg)	Diabetes/ Impaired glucose tolerance	tr Diabetes/IGT	Psychiatric disturbances	Osteoporosis	basal BMI (kg/m^2)	tr BMI (kg/m^2)
1	27	33	25	28	31	26	212	208	96	88	170/120	135/80	no	no	yes	osteoporosis	33.5	31.1
2	25	22	23	22	65	41	175	191	158	156	140/100*	135/80*	no	no	no	no	41.24	38.13
3	17	21	31	28	43	33	247	223	86	174	150/100	140/90	no	no	no	no	38	35.8
4	28	25	31	20	22	13	169	149	82	60	120/80	100/60	no	no	no	no	22	20.7
5	35	21	**73**	**18**	**158**	**50**	249*	222*	192	177	150/100*	130/80*	no	no	no	osteoporosis	38	37.2
6	**76**	**47**	**78**	**52**	**345**	**251**	**262***	**183**	**350**	**198**	125/90	120/85	DM (Ins 30 UI)	DM (Ins 20 UI)	yes	osteoporosis	32.98	30.3
7	31	24	20	22	45	41	161	126	110	87	125/70	115/70	no	no	no	no	40.33	35.2
8	31	22	52	36	52	76	184	182	68	96	130/80	110/70	no	no	no	no	32.88	32.88
9	14	19	24	26	46	35	198*	181*	176	248	160/90*	130/70*	IGT	no	yes	osteopenia	28	27.5
10	16	20	36	26	28	42	189	224	286	336	135/100*	130/90*	IGT	no	no	no	39.14	40.27
11	63	50	107	88	22	20	164	160	192	178	90/60	80/60	no	no	yes	no	44.9	44.9
12	23	14	34	28	52	68	257	193	202	370	160/90*	120/80*	no	no	yes	osteopenia	36.28	25.88

DISCUSSION

A 24-h urinary free cortisol (UFC) measurement is the most common means of monitoring disease activity in patients with CS (12), but it has a variety of significant limitations. It relies on a complete collection and, not surprisingly, this is often not the case and results in underestimation of disease severity. Another aspect is that the risk of effective medical treatment is overtreatment, inducing hypoadrenalism, which will go unrecognized with UFC measurement (4). Although more labor intensive, the measurement of serum cortisol is a more appropriate means of assessing disease activity. The best validated technique is the calculation of a mean serum cortisol from multiple measurements during a single day. Studies comparing isotopically determined cortisol production rates to serum levels indicate that a mean serum cortisol in the range 54-108 ng/mL equates to a normal cortisol production rate, and this should be the target of medical therapy (14). In our service dosage of UFC was not currently available, so in our retrospective study we had only the serum cortisol as marker of disease activity. As the highest value of cortisol is in the morning, and in CS there is no significant circadian variation of cortisol levels, we monitored the efficacy of treatment with the level of morning cortisol, and considered as controlled the patients who reached normal morning cortisol values (n.r. 70-225 ng/mL). This is a weakness of our study, but

the fact that the results are similar to those obtained using UFC suggests that the use of morning cortisol could have a comparable value. In our series, we obtained a 66% success rate in controlling hypercortisolism with Ketoconazole treatment. The mean time needed for normalization of cortisol level was 8.5 weeks. It is difficult to compare the results with other studies in the literature evaluating efficacy of medical therapy, because there are differences in patient's characteristics, previous treatment, medication type and doses, length of follow-up and criteria used to define disease control. Nonetheless, it is reported a 30–90% remission rate in Cushing's disease, with even higher rates being obtained in Cushing's syndrome of adrenal origin (1,8). A retrospective analysis (8) found normalization of UFC in 51.5 % of patients with CD in a mean follow-up of 23 months. The control of the disease was obtained in one to 3 months of therapy. Another recent review concluded that Ketoconazole is effective in approximately 50% of patients with ectopic ACTH secretion, as well as almost all patients with CS secondary to adrenal adenoma, but is rarely effective in patients with adrenocortical carcinoma (15). One recent study published by a French group enrolling 200 patients from 14 centers (the largest number ever reported concerning Ketoconazole efficacy in CD) reported 49.3% of patients with normalization of UFC and another 25.6% with a decrease more than 50% of UFC levels with Ketoconazole treatment (10).

Only one patient in our study presented the "escape syndrome". He had Cushing's disease due to a macroadenoma persistent after two surgeries and radiotherapy. Initially he responded well to Ketoconazole treatment, with the normalization of the cortisol level in one month, reflected by substantial clinical and biological improvement, but after another month he presented "escapement" from the control and we could not reestablish the control despite increasing the dose at 1000 mg/day, in consequence the patient was referred for bilateral adrenalectomy. Even in this case, the Ketoconazole treatment was beneficial, improving the biological parameters before surgery.

This low incidence of the "escape syndrome" is in accordance with previous studies (5, 10, 16). The reason for this escape from the drug effect is still not clear. Some authors claim that lowering cortisol in Cushing's disease patients may induce an ACTH response by the pituitary adenoma with the risk of escape from treatment efficacy (9). Others found that Ketoconazole may have direct effects on corticotrophic tumor cells in patients with Cushing's disease, inhibiting cell growth and ACTH production, effects that may explain the absence of a compensatory rise in ACTH levels in patients with Cushing's disease during prolonged treatment with Ketoconazole. (1, 17, 18). The latest studies showed that, in general, ACTH concentrations do not increase during long term treatment with Ketoconazole (3), consistent with our results.

In a large study about Ketoconazole in CD the authors have not found any significant difference between patients with or without a previous pituitary surgery, this point being particularly important in the patients with no visible adenoma on MRI (8). In our study, two patients, very well controlled with Ketoconazole, are still continuing the treatment, waiting that an adenoma could eventually be visualized and finally be cured by trans-sphenoidal surgery. Sustained remission of hypercortisolism was also obtained with steroidogenesis inhibitors in ectopic ACTH secretion (19) similar to the case reported in our study, which showed a very good response, not only on cortisol levels but also on ACTH levels.

Analyzing the group of patients uncontrolled with Ketoconazole, we conclude that the treatment was incorrectly conducted, with too low doses or too short time. None of these patients were treated with maximal doses, despite a good tolerance, which suggests a lack of confidence in the capabilities of the drug.

Concomitant with the cortisol decrease, an improvement of serum lipids, glycemic control and weigh was noted in our study, with no statistically significant difference, probably due to the small number of patients. Larger studies (10) confirm a good impact of Ketoconazole treatment upon hypertension and glycemic control.

The most significant issue with Ketoconazole treatment is a potentially increased risk of hepatotoxicity. Despite that bad reputation, studies enrolling patients with CS treated with Ketoconazole show a different picture: mild and transient elevations in liver enzymes of up to threefold of normal are occurring in some patients and are not a contraindication for further treatment (4, 9, 20, 21). The fact that none of our patients showed increase in the liver enzymes is surprising and discordant with previous data. The largest study (10) reports 18.4% of patients with increase in liver enzymes, from whom 85% had less than 5 fold increase, and liver enzymes returned to normal levels within 1 to 2 weeks after a dose decrease of 200 mg/day or treatment withdrawal. Our results are possibly due to the fact that none of our patients were treated with maximal doses. More intriguing are the two patients with basal elevated levels, who showed improvement of the liver enzymes during the treatment. Both were diagnosed with nonalcoholic steatohepatitis and maybe the improvement of the lipid levels during the

treatment was responsible for the decrease of the liver enzymes.

The impact on testosterone levels was minimal in men (as part of them was already deficient due to previous treatments at the pituitary level), but in men with normal level it must be considered and substitutive treatment implemented when needed. In women the elevated levels of testosterone were normalized in all patients, which is a supplemental beneficial effect.

The cortisol level has also to be closely monitored during the treatment, not only for the detection of the "escape syndrome", which can often be corrected with a higher dose, but also for the possibility to induce adrenal insufficiency that requires dose reduction The French study reported the first case of adrenal insufficiency during the treatment with Ketoconazole for hypercortisolism (10) and we report the second. In both cases, cortisol level was normalized by the reduction of doses, but the possibility of adrenal insufficiency makes obvious the need of a close monitoring of these patients, in specialized centers. In such conditions, the treatment can be safely conducted and the threats evoked can be diminished.

The safety of the treatment was also shown in other studies, where the side effects were absent or rare and often transitory, spontaneously or after lowering the dose (6, 10, 16, 21-23).

Although efficiency and safety of Ketoconazole have been demonstrated in several previous studies, this is the first paper concerning a series of treated Cushing's syndrome patients in Romania, confirming that Ketoconazole is efficient in diminishing hypercortisolism, with minimal side effects which are surpassed by its benefice. The present study has some limitations due to the small studied group, to his retrospective nature and to the use of serum cortisol level as parameter. We agree that a large, multicentric prospective study is needed in order to confirm this data, but first Ketoconazole must be maintained on the market for this particular and rare indication.

In conclusion, in our series of 12 patients, Ketoconazole therapy were well tolerated and were effective in most patients with Cushing's syndrome even in long term use. It should be maintained in the drug panoply of this severe disease. The resistance and the "escape syndrome" are possible, but rare, all patients requiring close monitoring during therapy. It can be considered as a primary medical therapy in patients without a visible pituitary adenoma and in patients with a high surgical risk caused by old age and/or significant comorbidities.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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