Terzolo M[1], Reimondo G, Chiodini I, Castello R, Giordano R, Ciccarelli E, Limone P, Crivellaro C, Martinelli I, Montini M, Disoteo O, Ambrosi B, Lanzi R, Arosio M, Senni S, Balestrieri A, Solaroli E, Madeo B, De Giovanni R, Strollo F, Battista R, Scorsone A, Giagulli VA, Collura D, Scillitani A, Cozzi R, Faustini-Fustini M, Pia A, Rinaldi R, Allasino B, Peraga G, Tassone F, Garofalo P, Papini E, Borretta G.

Screening of Cushing's syndrome in outpatients with type 2 diabetes: results of a prospective multicentric study in Italy

J Clin Endocrinol Metab.; 97(10); 2012; 3467-75. DOI: 10.1210/jc.2012-1323.

SCREENING OF CUSHING'S SYNDROME IN A LARGE SERIES OF DIABETIC PATIENTS IN AN OUTPATIENT SETTING.

M. Terzolo[1], G. Reimondo[1], I. Chiodini[2], R. Castello[3], R. Giordano[4], E. Ciccarelli[5], P. Limone[6], C. Crivellaro[7], I. Martinelli[8], M. Montini[9], O. Disoteo[10], B. Ambrosi[11], R. Lanzi[12], M. Arosio[13], S. Senni14, A. Balestrieri, E. Solaroli[15], A. Madeo, R. De Giovanni16, F. Strollo17, R. Battista18, A. Scorsone19, V.A. Giagulli[20], D. Collura21, A. Scillitani22, R. Cozzi23, M. Faustini-Fustini24, A. Pia[25], R. Rinaldi[27], B. Allasino[1], G. Peraga1, F. Tassone[25], P. Garofalo[26], E. Papini[27], G. Borretta[25].

[1] Medicina Interna I, A.O.U. San Luigi Gonzaga, Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Torino; [2]Endocrinologia e Diabetologia, Ospedale Maggiore Policlinico IRCCS, Università di Milano, Milano; [4]Endocrinologia, Diabetologia e Metabolismo, AOU Molinette, Dipartimento di Medicina Interna, Università di Torino, Torino; [5]S.S. Patologie Endocrine e Metaboliche, Ospedale E. Valdese, Torino; [7]Endocrinologia, Dipartimento di Medicina Interna, Ospedale Centrale di Bolzano, Bolzano; [9]Endocrinologia, Ospedali Riuniti di Bergamo, Bergamo; [10]Diabetologia, A.O. Ospedale Niguarda, Milano; [11]Endocrinologia e Diabetologia, IRCCS Policlinico San Donato, Dipartimento di Scienze Medico-Chirurgiche, Università di Milano, Milano; [12]Endocrinologia, Dipartimento di Medicina Interna e Specialistica, IRCCS

Fondazione Centro San Raffaele del Monte Tabor, Milano; [13]Endocrinologia e Diabetologia, Ospedale San Giuseppe, Dipartimento di Scienze Mediche, Università di Milano, Milano; [15]Endocrinologia, Ospedale Maggiore Bellaria, Bologna; [20]Malattie Metaboliche ed Endocrinologia, P.O. Monopoli-Conversano, Bari; [25]Endocrinologia, A.O. S. Croce e Carle, Cuneo; [26]Endocrinologia dell'Età Evolutiva, Ospedale Cervello, Palermo; [27]Endocrinologia e Malattie Metaboliche, Ospedale Regina Apostolurum, Albano Laziale; Italy.

Running title: screening of Cushing in diabetes.

Key words: cortisol, Cushing's syndrome, dexamethasone test, diabetes mellitus.

Address correspondence to:

M. Terzolo, MD
Medicina Interna I
A.O.U. San Luigi Gonzaga
Regione Gonzole 10
10043 Orbassano ITALY
Tel: ++ 39 011 9026788
Fax: ++ 39 011 6705456
e-mail: terzolo@usa.net

INTRODUCTION

Subtle clinical presentations of Cushing's syndrome, characterized by a paucity of signs and symptoms due to mild cortisol hypersecretion, are increasingly found in clinical practice. In parallel, the metabolic syndrome epidemic is leading to a boost in the number of patients with a Cushingoid phenotype, who could be potentially candidate to be tested for hypercortisolism (1).

The Endocrine Society guidelines for the diagnosis of Cushing's syndrome recommended against widespread testing for the condition, unless in patients with unusual features for age, or multiple and progressive features, particularly those that are more predictive of Cushing's syndrome (2). However, unsuspected Cushing's syndrome was found to be more frequent than previously expected in different series of diabetic patients submitted to routine screening (3, 4, 5). Thus, a number of patients with Cushing's syndrome may not be recognized while they are managed for diabetes, either because of a mild clinical presentation or because of insufficient awareness of their physicians. Missing a diagnosis of Cushing's syndrome may have detrimental consequences on patient outcome because hypercortisolism is expected to worsen metabolic control and increase the probability of future cardiovascular events (6).

The results of the above-mentioned studies may argue in favor of a systematic screening of Cushing's syndrome in type 2 diabetes. However, data were mostly generated in academic centers on hospitalized patients, thus raising the possibility of a selection bias toward patients at greater risk of Cushing's syndrome than the general diabetic population (7).

The aim of our study was to screen patients attending diabetes clinics in an outpatient setting and in conditions of standard clinical practice. Cohorts of

consecutive patients were enrolled in different Italian diabetes clinics rather than selecting groups of patients at higher risk for Cushing's, i.e. those who were hypertensive, overweight, and had poor glycemic control.

PATIENTS and METHODS

Patients

The study was conducted under the auspices of the Associazione Medici Endocrinologi (AME) at 24 diabetes clinics across Italy (Fig/table 1). Written informed consent was obtained from all patients, and the institutional review board at each center approved the study. Each participating center was requested to recruit consecutively at least 20 diabetic patients fulfilling all inclusion criteria and without exclusion criteria, who were attending the center for an ordinary outpatient visit from June 2006 to April 2008. Follow-up for this study was closed in September 2010. Patients had to meet the following inclusion criteria: age between 18 and 70 years, BMI greater than 25 kg/m^2 and known diagnosis of type 2 diabetes on active follow-up from at least 1 year before the study. Exclusion criteria were presence of specific Cushingoid features (easy bruising, facial plethora, proximal miopathy and striae (2)), any severe acute illness, treatment with drugs known to affect the HPA axis or dexamethasone metabolism, current or previous history of alcohol abuse or major mood disorders that required psychiatric intervention, history of recent surgery or trauma and pregnancy. Any subject with BMI greater than 30 kg/m^2 was categorized as obese (8). Any subject with systolic blood pressure greater than 140 mmHg, diastolic blood pressure greater than 90 mmHg, or on antihypertensive treatment was categorized as hypertensive (9). Dyslipidemia was defined following the ATP-III criteria: total cholesterol >200 mg/dl, high-density lipoprotein cholesterol levels <40 mg/dl, serum triglyceride levels >150 mg/dl, low-density lipoprotein cholesterol levels >160 mg/dl (10). Patients were also considered dyslipidemic if any specific treatment was given. The main characteristics of the evaluated patients are shown in Table 1.

Materials and protocols

All subjects underwent in an outpatient setting a first screening step by using the overnight 1-mg dexamethasone suppression test (DST) (1 mg dexamethasone administered orally at 2300 h, and blood sample drawing on the following morning at 0800 h for determination of serum cortisol concentration. Patients who failed to suppress serum cortisol less than 5.0 μg/dl (138 nmol/l) were offered a second-step evaluation by undergoing a standard 2-day 2-mg DST, 3–6 months after baseline evaluation (0.5 mg dexamethasone administered orally at 06.00, 12.00, 18.00 and 24.00 h, and blood sample drawing on the following morning at 0800 h for cortisol determination). A cortisol concentration greater than 1.8 μg/dl (50 nmol/l) was considered abnormal and prompted further evaluation to confirm diagnosis of Cushing's syndrome and determine its cause according to a standardized protocol (11). The study flow-chart is outlined in Figure 1. Briefly, evaluation included 24-h urine collection for urinary free cortisol measurement and blood drawing at 0800 h for determination of plasma ACTH concentration. ACTH concentration provided guidance for radiologic evaluation (pituitary MRI or adrenal CT); in doubtful cases, the corticotropin-releasing hormone (CRH) stimulation test was also performed to ascertain the ACTH dependency when ACTH concentration was between 10 and 20 pg/ml (2.2 and 4.4 pmol/l) (12). Specific treatment was pursued in the patients with a definitive diagnosis of Cushing's syndrome who were followed up for at least 24 months after surgery. The hormonal variables were determined in a single reference laboratory for each participating center using commercially available reagents.

Statistical analysis

Sample size analysis was done based on the results of previous studies. It was calculated that approximately 381 patients should be studied to provide an 80% chance (beta) of detecting a prevalence rate of Cushing's syndrome of 1%, taking 0.05 as the level of significance (alpha). Rates and proportions were calculated for categorical data, and means and standard deviations for continuous data. Normality of data was assessed by the Kolmogorov-Smirnov test. For continuous variables, differences were analyzed by means of the two-tailed Student's *t*-test when data were normally distributed and by the Mann–Whitney *U*-test for nonparametric data. For categorical variables, differences were analyzed by means of the $\chi 2$-test and Fisher's exact test. Levels of statistical significance were set at $P < 0.05$. A multiple regression analysis was performed when appropriate. All analyses were performed using the Statistica® software package (Microsoft Corp, Tucla, OK, USA).

RESULTS

A total of 813 consecutive patients with type 2 diabetes were enrolled. They were 428 men (52.6%) and 385 women (47.4%) aged 25–70 years (median, 60 years) with a median duration of diabetes of 8 years (range, 1-20 years). Of the whole cohort, 17.9% of patients were treated with insulin, 62.2% with oral hypoglycemic agents, 13.7% with a combined therapy, while 6.2% were on diet alone (patients on glitazones were excluded from the study because these drugs reportedly interfere with the HPA axis (13)). Overall, 71.7% of patients were hypertensives, 56.5% of whom were treated with 2 or more drugs, 58.7% had LDL cholesterol >100 mg/dl, and 79.9% were dyslipidemic, 46.8% of whom were on pharmacologic treatment.

All subjects underwent a first screening step for Cushing's syndrome using the overnight 1-mg DST. In a multiple regression analysis including as candidate predictive variables age, BMI, systolic and diastolic blood pressure, glycosilated hemoglobin (HbA1c) and fasting glucose levels, post-DST cortisol levels were associated with systolic blood pressure (β=0.09, P=0.02) and HbA1c values (β=0.12, P=0.001). However, the model accounted only for 2% (r^2) of the total variation (P=0.007). The patients with HbA1c >7% had post-DST cortisol levels higher than the remainders (1.73 ± 2.28 μg/dL vs 1.36 ± 1.23 μg/dL, [48 ± 63 nmol/l vs 38 ± 34 nmol/l] p=0.02).

Forty patients (4.9%) failed to suppress cortisol <5.0 μg/dl (138 nmol/l) after the 1-mg DST. They were 21 men and 19 women aged 20-70 years (median, 56.5 years). The patients failing to suppress cortisol were slightly younger with similar duration of disease and BMI compared to the remainders. There was no difference in terms of treatment for either hypertension or dyslipidemia, while a greater percentage of non-suppressing patients was on insulin treatment (51.3% vs 30.4%, p=0.01). Moreover, the non-suppressing patients showed greater systolic blood pressure, HbA1c and

fasting glucose levels than the patients with post-DST cortisol <5 μg/dL (138 nmol/l) (Table 2).

The 40 non-suppressing patients underwent a second-step evaluation with a standard 2-day 2-mg DST (2). Thirty-four patients (85%) displayed cortisol levels <1.8 μg/dl (50 nmol/l) and were considered as false-positives after the 1-mg DST, while the diagnosis of Cushing's syndrome was confirmed in 6 patients (0.7% of the whole series) (Table 3). In 5 patients, adrenal-dependent Cushing's syndrome was diagnosed on the basis of undetectable ACTH concentrations in four of them (cases #1, 2, 3, 5) and undetermined ACTH not responsive to the CRH test (basal ACTH 10 pg/ml [2.2 pmol/l], peak ACTH 12 pg/ml [2.6 pmol/l] in patient #4). Computerized tomography (CT) scan was then performed and an adrenal tumor was found in 4 patients (cases #1, 2, 3, 5) while bilateral macronodular adrenal hyperplasia was observed in patient #4. The patient with normal ACTH levels (case #6) underwent pituitary magnetic resonance imaging (MRI) that did not show any lesion and the patient refused further investigation (Table 3).

Three out of 6 patients with a definitive diagnosis of Cushing's syndrome had less than 50 years (cases #1, 3, 5). Only one patient had HbA1c values <7.0% (case #6) and 3 patients had poor glycemic control despite intensive treatment (cases #1, 2, 3). Four patients had a difficult to treat hypertension (cases #1, 2, 5, 6). The patients bearing a cortical adenoma underwent adrenalectomy and the pathological diagnosis was adrenal adenoma. Twelve months after adrenalectomy, an eucortisolemic state was restored in these patients who were able to discontinue all glucose-lowering agents in 3 of them (cases #1, 2, 5) and insulin in another (case #2), attaining adequate metabolic control; in one patient (case #5) also anti-hypertensive treatment could be stopped. The patient with ACTH-independent bilateral macronodular hyperplasia (case #4) did not undergo surgery because of poor clinical

conditions.

DISCUSSION

Previous studies have shown a prevalence of unsuspected Cushing's syndrome in patients with type 2 diabetes ranging from 0% to 9.4%, even if surgical prove was secured in a minority of cases only (Table 4). The studies reporting the highest prevalence figures included patients with some features conferring an elevated a priori probability of Cushing's syndrome (such as obesity, hypertension and poor diabetes control, which were often concomitantly present). Moreover, these studies were done in hospitalized patients only. The analysis of the available literature suggests that either the study setting (inpatient versus outpatient), the patient characteristics and the test cutoff are important factors determining the outcome of the screening (Table 4).

In the present study, we observed a frequency of previously unsuspected Cushing's syndrome of 0.7% in a cohort of 813 patients with type 2 diabetes, the largest series up to now reported. Although this figure is low in absolute terms to support the efficacy of a widespread screening, it makes Cushing's syndrome much more frequent than previously thought considering that type 2 diabetes affects about 4% of the adult population in Italy (14). The present findings add to previous evidence showing that occult Cushing's syndrome recognizes more frequently an adrenal etiology, whereas overt Cushing's syndrome is more commonly pituitary-dependent (15). These data suggest that most cases of Cushing's syndrome may actually be unrecognized because of phenotypic similarities with the metabolic syndrome (1).

Strengths of our study include the large number of patients, the multicentric nature and the outpatient setting of the study, making our results generalizable to an unselected patient population. In fact, our patients attending the diabetic care units involved in the study are fully representative of the general diabetic population in Italy because they present similar

demographic, clinical and biochemical features to those reported in the yearly surveys on type 2 diabetes in Italy (14).

The frequency of occult Cushing's syndrome reported herein was lower than in previous studies employing a similar screening strategy (3, 4, 5) and this is likely explained by two factors. First, in our study screening was done in an unselected population and not only in patients at perceived high risk of hypercortisolism. It is pertinent to consider that different procedures of screening proved to be ineffective in an outpatient setting, although the studies had less statistical power than the present one (16, 17, 18, 19). Second, the use of a more specific cutoff point for the 1-mg DST at 5.0 μg/dl (138 nmol/l). This was deemed appropriate since the study was done in conditions of standard clinical practice and not in academic, referral centers. Our choice was aimed at limiting additional workload and costs of screening to select patients with more than minimal hypercortisolism, likely associated with clinical consequences. The entity of hypercortisolism was indeed slight in one case only, with a presumably pituitary adenoma.

In previous studies, the use of cutoff points ranging from 1.8 μg/dl (50 nmol/l) to 2.1 μg/dl (58 nmol/l) was associated with a high false-positive rate due to the poor specificity of these thresholds (3, 4, 20), whereas a cutoff point at 4.0 μg/dl (110 nmol/l) provided comparable results (21). Would have we used the most sensitive cutoff of 1.8 μg/dl (50 nmol/l), as many as 22.6% of patients should have been submitted to further testing. This observation demonstrates clearly that a screening strategy aimed at maximal sensitivity, that should be advocated in principle, is virtually impossible to apply in the every-day practice. However, we cannot exclude to have missed a number of patients with subclinical Cushing's syndrome, that is associated with subtler alterations of the HPA axis (22). This observation does not limit the clinical relevance of our results, since it remains controversial whether subclinical

Cushing's syndrome is associated with long-term morbidity and whether treatment to reverse this disorder may be beneficial (7, 22, 23).

Two issues are key to the justification of large-scale screening of Cushing's syndrome. First, is occult Cushing's syndrome associated with a more severe metabolic and cardiovascular disease and, second, does its cure have a beneficial impact on the outcome of patients? These issues remain mostly undefined by the available studies, because of the small number of patients who were found to have Cushing and were submitted to specific treatment (Table 4). In addition, none of the previous studies reported on long-term data after successful treatment of occult Cushing's syndrome. Discrepant results were reported concerning severity of diabetes in occult Cushing (3, 4); however, amelioration of diabetes has been reported in the few patients who attained remission of hypercortisolism (3, 4, 21).

In the present study, we observed that the condition of being non-suppressor to dexamethasone was associated with higher glycemic levels, notwithstanding that a greater proportion of such patients were on insulin treatment. However, this association seems to reflect a secondary activation of the HPA axis in patients with more severe metabolic derangement (24, 25), since we observed a positive relationship between HbA1c values and post-dexamethasone cortisol levels.

We were able to attain long-term follow-up data of the patients with definitive Cushing's syndrome, thus confirming that surgical cure was associated with significant improvement of metabolic control in the 4 patients who underwent removal of their adrenal adenoma (Table 3). Diagnosis of Cushing's syndrome was useless in a patient in poor general conditions, who is still untreated. Due to the small numbers, we cannot definitively prove that screening of Cushing's syndrome results in a more favorable outcome of the patients who were diagnosed with the condition. However, it is noteworthy

that all the treated patients were able to discontinue, or reduce, medications for diabetes after remission of hypercortisolism.

In conclusion, the results of the present study do not support the application of a wide-scale screening of Cushing's syndrome in patients with type 2 diabetes, unless more efficient screening procedures will become available. The frequency of Cushing's syndrome in an unselected patient population was low compared to the number of false positive results to make a routine screening strategy applicable in practice.

Considering the epidemic of type 2 diabetes in the Western World, however, the present data suggest that Cushing's syndrome is less rare than previously found (although not frequent enough to warrant systematic screening). This is plausible since the available epidemiological data took into account only diagnoses made in hospitalized patients, thus considering only the most severe and clinically obvious cases (26, 27, 28, 29).

Our results may influence clinical practice supporting a case-finding approach in selected cases. The characteristics of our group of patients with a definitive diagnosis of Cushing's suggest that a difficult control of diabetes (and hypertension) despite intensive treatment should prompt screening, particularly when features suggestive of hypercortisolism are apparent (1, 2). A diagnosis of type 2 diabetes below 50 years of age is another factor that should raise suspect, recalling the value of features unusual for age to suspect Cushing (2), since the disease usually occurs in older patients (14). However, age at diagnosis of type 2 diabetes is shifting among younger population (30). The patients found to have occult Cushing's syndrome should be referred to endocrinologists with specific expertise to ensure a prompt treatment of the condition that may have a beneficial impact on health outcomes. It is pertinent to consider that adrenal adenoma is the leading cause of occult Cushing's syndrome and the fact that laparoscopic

adrenalectomy is become a safe and relatively inexpensive procedure, causing a limited discomfort to the patient (31), makes more attractive the search for the condition. Since patients with occult Cushing's syndrome have a milder clinical phenotype, physicians involved in management of diabetes should raise their level of awareness for the condition. The knowledge and experience of the physician is key in this context, thus the present data suggest that a specific educational policy may lead to an improved care of patients with type 2 diabetes.

REFERENCES

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001 Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 285:2486–2497

Voce Francesco: Changes in age at diagnosis of type 2 diabetes mellitus in the United States, 1988 to 2000. Koopman RJ et al. Ann Fam Med. (2005)

Table 3 Characteristics of the patients with definitive Cushing's syndrome. Ricontrollare l'outcome dei pazienti e dare UFC

#	Sex	Age (yrs)	BMI (kg/m²)	HbA₁C (%)	T2DM duration (yrs)	T2DM therapy	HTN therapy (n° of drugs)	Cortisol post DST (µg/dl)	Cortisol post Liddle I (µg/dl)	UFC (µg/24h)	ACTH (pg/ml)	Imaging test	Tumor found (size [mm] / side)	Patient outcome
1	M	32	44.9	11.0	5	OHA + I	3	20.8	13.7		<5	Adrenal CT	25/right	Treatment of T2DM stopped after ADX
2	F	69	57.3	10.6	11	OHA + I	6	12.6	2.1		<5	Adrenal CT	32/right	Deceased after xx months
3	M	20	28.0	12.0	2	OHA + I	0	16.5	12.1		<5	Adrenal CT	28/left	Treatment of T2DM stopped after ADX
4	M	68	32.8	7.8	20	I	2	13.3	2.8		10	Adrenal CT	BMAH	??
5	M	44	26.7	7.8	4	OHA	5	23.3	20.0		<5	Adrenal CT	31/left	Treatment of T2DM and HTN stopped after ADX
6	F	67	26.0	6.4	1	OHA	3	6.2	17.4		22.9	Pituitary Pituitary MRI	-	??

Abbreviations are as follows: T2DM, type 2 diabetes; HTN, hypertension; DST, dexamethasone suppression test; OHA, oral hypoglycemic agents; I, insulin; CT, computerized tomography; ADX, adrenalectomy; BMAH, bilateral macronodular adrenal hyperplasia; MRI, magnetic resonance imaging.

Figure1 Algorithm of the screening strategy used in 813 patients with type-2 diabetes.

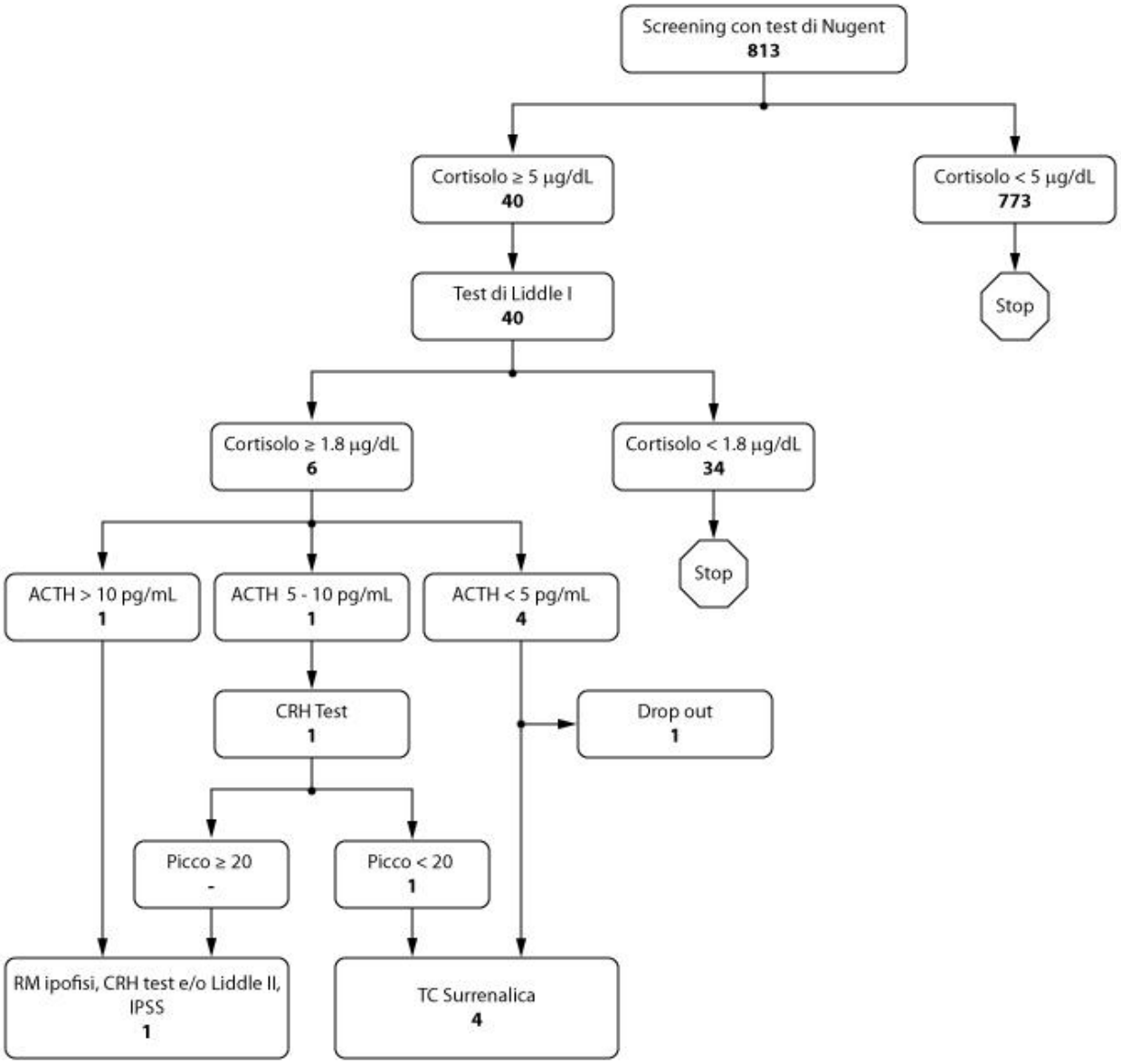

Table 1 Main characteristics of the patients.
Data are expressed as mean values and standard deviations.

Variable	
Age [years]	58.9 ± 8.9
BMI [kg/m^2]	32.1 ± 6.1
Duration of disease [years]	9.8 ± 7.9
Fasting glucose [mg/dl]	174.7 ± 67.7
HbA_1C [%]	8.4 ± 1.9
Systolic blood pressure [mmHg]	138.2 ± 17.2
Diastolic blood pressure [mmHg]	82.6 ± 10.1
Total cholesterol [mg/dl]	186.9 ± 48.7
HDL cholesterol [mg/dl]	45.9 ± 23.7
LDL cholesterol [mg/dl]	106.9 ± 45.6
Triglycerides [mg/dl]	171.2 ± 157.2

Table 2 Comparison of patients with post-DST cortisol ≤5 μg/dl (DST suppressors) and post-DST cortisol >5 μg/dl (DST non-suppressors). Data are expressed as mean values and standard deviations.

	DST suppressors	**DST non-suppressors**	**p**
Age [years]	58.6 ± 8.8	55.5 ± 11.9	0.03
BMI [kg/m^2]	32.1 ± 6.1	31.7 ± 6.4	NS
Duration of disease [years]	9.8 ± 7.9	8.8 ± 5.7	NS
Fasting glucose [mg/dl]	172.5 ± 65.1	222.9 ± 100.7	<0.0001
HbA_1C [%]	8.4 ± 1.9	9.3 ± 2.0	0.005
Systolic blood pressure [mmHg]	137.9 ± 17.2	147.1 ± 16.9	0.02
Diastolic blood pressure [mmHg]	82.6 ± 10.1	80.7 ± 10.9	NS

REFERENCES

1. Findling
2. **Nieman LK, Biller BMK, Findling JW, Newell-Price J, Savage MO, Stewart PM, et al**. The Diagnosis of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2008; 93(5):1526-1540.
3. **Chiodini I, Torlontano M, Scillitani A, Arosio M, Bacci S, Di Lembo S, et al**. Association of subclinical hypercortisolism with type 2 diabetes mellitus: a case-control study in hospitalized patients; European Journal of Endocrinology. 2005; 153 837-844.
4. **Catargi B, Rigalleu V, Poussin A, Ronci-Chaix N, Bex V, Vergnot V, et al**. Occult Cushing's Syndrome in Type-2 Diabetes. J Clin Endocrinol Metab. 2003; 88(12):5808-5813.
5. **Leibowitz G, Tsur A, Chayen SD, Salameh M, Raz I, Cesari E, et al**. Pre-clinical Cushing's syndrome: an unexpected frequent cause of poor glycaemic control in obese diabetic patients. Clinical Endocrinology. 1996; 44, 717-722.
6. **Boscaro M, Barzon L, Fallo F, Sonino N**. Cushing's syndrome. The Lancet. 2011; Marzo 10, Vol 357.
7. **Chiodini I.** Clinical review: Diagnosis and treatment of subclinical hypercortisolism. J Clin Endocrinol Metab. 2011; 96(5):1223-36.
8. 8 clinendo
9. 9 clinendo
10. VOCE APT III
11. 7 clinendo
12. **Reimondo G, Pia A, Bovio S, All'asino B, Daffara F, Paccotti P, Borretta G, et al**. Laboratory differentiation of Cushing's syndrome. Clinica Chimica. 2008; Acta 388: 5–14.
13. VOCE
14. http://infodiabetes.it/files/ANNALI2010.pdf (verificare indirizzo e mettere quando è stato visitato)
15. Tabarin review
16. **Liu H, Bravata DM, Cabaccan J, Raff H and Ryzen E**. Elevated late-night salivary cortisol levels in elderly male type 2 diabetic veterans. Clinical Endocrinology. 2005; 63, 642–649.
17. **Mullan K, Black N, Thiraviaraj A, Bell PM, Burgess C, Hunter SJ, et al**. Is There Value in Routine Screening for Cushing's Syndrome in Patients with Diabetes?. J Clin Endocrinol Metab. 2010; 95(5):2262–2265.
18. **Newsome S, Chen K, Hoang J, Wilson JD, Potter JM and Hickman PE**. Cushing's syndrome in a clinic population with diabetes. Internal Medicine Journal. 2008; 38: 178–182.
19. GAGLIARDI
20. **Caetano MSS, Do Carmo Silva R, Kater CE**. Increased Diagnostic Probability of Subclinical Cushing's Syndrome in a Population Sample of Overweight Adult Patients with Type 2 Diabetes Mellitus. Arq Bras Endocrinol Metab. 2007; 51/7.
21. **Reimondo G, Pia A, All'asino B, Tassone F, Bovio S, Borretta G, et al**. Screening of Cushing's syndrome in adult patients with newly diagnosed diabetes mellitus. Clinical Endocrinology. 2007; 67, 225-229.
22. **Terzolo M, Reimondo G, Bovio S, Angeli A**. Subclinical Cushing's Syndrome. Pituitari. 2004; 7: 217–223.
23. GRUMBACH
24. 28, 87-89 di review tabarin
25. **Chiodini I., Adda G., Scillitani A., Coletti F., Morelli V., Di Lembo S., et al**. Cortisol secretion in patients with type 2 diabetes: relationship with chronic complications. Diabetes Care. 2007; 30(1):83-88.
26. **Lindholm**
27. **Extabe**
28. **Clayton**
29. **Bolland**
30. **Koopman RJ, et al**. Changes in age at diagnosis of type 2 diabetes mellitus in the United States, 1998 to 2000. Ann Fam Med. 2005.
31. VOCE REVIEW