RESEARCH

Cardiovascular risk factors in mild adrenal autonomous cortisol secretion in a Caucasian population

Agnieszka Adamska[1], Vitalii Ulychnyi[1], Katarzyna Siewko[1], Anna Popławska-Kita[1], Małgorzata Szelachowska[1], Marcin Adamski[2], Angelika Buczyńska[3] and Adam Jacek Krętowski[1,3]

[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Białystok, Bialystok, Poland
[2]Faculty of Computer Science, Bialystok University of Technology, Białystok, Poland
[3]Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland

Correspondence should be addressed to A Adamska: ak001@wp.pl

Abstract

Cardiovascular risk factors could be present in mild adrenal autonomous cortisol secretion (MACS). However, the most frequent cardiovascular risk factors in MACS have not been established. The aim of the presseent study was to analyse the difference in cardiovascular risk factors in patients with MACS in comparison to those with non-functioning adrenal tumour (NFAT). A total of 295 patients with adrenal incidentaloma were included in this retrospective study. We divided our group into those who showed suppression in 1 mg overnight dexamethasone suppression test (DST) (NFAT) (serum cortisol level ≤ 1.8 μg/dL) and those who did not show suppression in the DST (MACS) (serum concentration of cortisol > 1.8 μg/dL and ≤ 5 μg/dL). In the studied groups, we analysed the presence of cardiovascular risk factors, such as obesity, prediabetes, type 2 diabetes mellitus (T2DM), hypertension, hyperlipidaemia, chronic kidney disease and cardiovascular events. In our study, 18.9% of patients were defined as MACS. Importantly, T2DM was diagnosed in 41% of MACS vs 23% of NFAT ($P < 0.01$) and higher frequency of occurrence of hyperlipidaemia in NFAT (72.4%) vs MACS (53.6%) ($P = 0.01$) was observed. We did not observed differences in the frequency of obesity, hypertension, chronic kidney disease, prediabetes, atrial fibrillation, stroke, ST and non-ST elevation myocardial infarction and coronary angioplasty between patients with MACS and NFAT (all $P > 0.05$; respectively). In MACS, T2DM is more prevalent than in NFAT; hyperlipidaemia is more prevalent in NFAT. Accordingly, no differences were found in the incidence of obesity, hypertension, prediabetes, chronic kidney disease between studied groups as well as cardiovascular events.

Introduction

An adrenal incidentaloma is defined as a lesion of the adrenal gland that is discovered incidentally during imaging techniques performed for disorders unrelated to the adrenal gland (1, 2). In most cases, adrenal incidentalomas are benign and not associated with clinically relevant hormonal hyperfunction (non-functioning adrenal tumour, NFAT); they are observed in <1% in young people, 3% in middle-aged adults and >15% in subjects over 70 years old (3, 4). However, pheochromocytoma, aldosteronism, or overt cortisol excess due to hyperfunction may be observed in patients with adrenal incidentaloma. It has been shown that cortical adenoma is the most frequent tumour type among adrenal incidentalomas (5), whereas adrenal incidentalomas with mild cortisol excess, when the classical clinical features of Cushing's syndrome are not present, are defined as mild

autonomous cortisol secretion (MACS) (6). It has been reported that MACS is observed in 15–50% of patients with adrenal incidentalomas (7, 8). The classical triad commonly used to define MACS is characterised by disturbance of the hypothalamus–pituitary–adrenal axis in patients with incidentally discovered adrenal masses who do not exhibit signs and symptoms specific to overt Cushing's syndrome (9). The definition of MACS is based on the serum cortisol values after a 1 mg overnight dexamethasone suppression test (DST); however, the cut-off values are not clearly established (9). In the European Society of Endocrinology (ESE) guidelines, a serum cortisol level of ≤50 nmol/L (1.8 μg/dL) in the DST is regarded to exclude hypercortisolism, values between 50 (1.8 μg/dL) and 138 nmol/L (5 μg/dL) are referred to as possible autonomous cortisol secretion and values of >138 nmol/L are termed autonomous cortisol secretion (3). Nevertheless, these guidelines underestimated a nowadays well-established concept, namely, that cortisol secretion in the adrenal gland follows a continuum from physiological to undoubtedly increased levels. Same authors recommended a lower cortisol level after DST, equal to 1.09 μg/dL, to exclude MACS (10). Therefore, any diagnostic cut-off is an arbitrary threshold possibly leads to misclassification of some patients. In consideration of this, a global patient evaluation including comorbidities and life expectancy is essential to reach treatment decisions (11).

The classic cardiovascular risk factors are obesity, type 2 diabetes mellitus (T2DM), prediabetes (PD), hypertension, hyperlipidaemia and chronic kidney disease (CKD). It has been shown that cardiovascular risk factors are present in patients with MACS; however, how to identify patients with MACS who would most likely benefit from adrenalectomy is not established (12). It has been shown that MACS is connected to an increased risk of T2DM, hypertension and hyperlipidaemia, as well as cardiovascular events and mortality (13, 14). Sbardella *et al.* showed that left ventricular mass index was increased in patients with MACS compared to NFAT (15). However, in the study conducted by Podbregar *et al.*, the patients with MACS did not differ in BMI, blood pressure, heart rate, lipid profile, fasting glucose and presence of T2DM when compared to NFAT (16). Interestingly, an impaired cardiovascular profile is a common finding in patients with apparent NFAT (17) and patients with NFAT can show a high prevalence of obesity, glucose intolerance, T2DM and hyperlipidaemia (8, 18, 19, 20). Moreover, it has been shown that NFAI was related with an increased risk of cardiovascular events and their related mortality occurrence (21).

In the face of the fact that it has been shown that the incidence of cardiovascular outcomes and related mortality seems to be increased in patients with MACS (22) and NFAT may be associated with increased cardiometabolic risk during follow-up (17). The aim of the present study was to analyse different cardiovascular risk factors in patients with MACS in comparison to those with NFAT.

Materials and methods

Subjects

In this study, we retrospectively analysed the records of 335 patients with adrenal incidentaloma referred to the Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Białystok, between January 2017 and June 2019. Among all patients hospitalised with adrenal incidentaloma in the studied time, 40 were not included in the study due to the exclusion criteria. A total of 295 patients with adrenal incidentaloma were included in this retrospective study. We excluded patients with alcoholism or psychiatric diseases; those taking drugs influencing cortisol and dexamethasone metabolism or cortisol secretion; those with signs or symptoms of overt cortisol excess (i.e. moon facies, striae rubrae, skin atrophy, or buffalo hump) and those with a serum cortisol level of more than 5 μg/dL in the DST, haemorrhage, infections, adrenal pheochromocytoma, primary hyperaldosteronism, metastases, adrenocortical carcinoma, cysts, history of malignancy or hyperparathyroidism.

According to ESE guidelines, MACS was diagnosed using the DST with cut-off values for the serum concentration of cortisol of >50 nmol/L (1.8 μg/dL) and ≤138 nmol/L (5 μg/dL) (3).

The study complied with the Declaration of Helsinki and was approved by the Ethical Committee of Białystok (no. APK.002.14.2022).

Study protocol

In all subjects, the following parameters were recorded: age, gender, presence of obesity, hypertension, T2DM, PD, CKD and hyperlipidaemia. The BMI was calculated as weight divided by height squared, and obesity was diagnosed when the BMI was higher than 30 kg/m². We also recorded the size of tumour of adrenal gland. If fasting blood glucose level was lower than 126 mg/dL, an oral glucose tolerance test (OGTT) with 75 g of glucose was performed in patients without known T2DM. Accordingly, patients undergoing

antidiabetic treatment were considered as having T2DM, thus OGTT were not performed. A prediabetes state was defined as impaired fasting glucose (fasting glucose of 100–125 mg/dL) and/or impaired glucose tolerance (serum glucose level of 140–199 mg/dL in the 2nd hour during the OGTT). Hypertension was defined as a documented diagnosis of hypertension treated with at least one antihypertensive medication. Hyperlipidaemia was defined as a triglyceride level of ≥150 mg/dL (1.7 mmol/L), or HDL cholesterol levels of <40 mg/dL (1.0 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women, or the presence of LDL) cholesterol level higher than 115 mg/dL (mmol/L), or if treatment was previously prescribed for hyperlipidaemia. CKD was recognised according to the guidelines of Improving Global Outcomes (KDIGO) (23).

In all patients, the plasma concentration of adrenocorticotrophic hormone (ACTH) at 08:00 h, 24 h urinary free cortisol (24 h UFC) measurements, cortisol in saliva at 24:00 h and 1 mg DST were determined.

In all patients, a CT scan was performed for unrelated diseases, that is, abdominal pain, back pain, or post-accident. All adrenal lesions displayed a CT pattern consistent with benign adenoma (i.e. homogeneous texture, less than 10 Hounsfield units and regular margins). Adrenal adenoma was confirmed in case of absolute washout of >60% or a relative washout of >40% in the delayed images (15 min for the venous phase) during contrast-enhanced CT (2).

Laboratory test

Serum and urinary cortisol levels and plasma ACTH concentrations were measured using commercially available reagents. The serum and saliva cortisol determinations were performed via the electrochemiluminescence immunoassay (ECLIA) method, intended for use on the Cobase immunoassay analyser E411 (Roche, Switzerland, 06687733), characterised by a sensitivity of 1.5 nmol/L, with an intra-assay coefficient of variation (CV) of 2%, and an inter-assay CV of 3.8% and an intra-assay CV of 2.5%, and an inter-assay CV of 3.6% evaluated for serum and saliva measurements, respectively.

The EDTA plasma ACTH concentration was measured via the ECLIA method (sensitivity, 1.00 pg/mL; intra-assay CV, 1.1%; inter-assay, CV 2.4%) performed with the use of a Roche E411 device (Roche Diagnostics, Sussex, UK, 03255751190). The UFC was determined via the chemiluminescent microparticle immunoassay (CMIA) method (sensitivity, ≤1 μg/dL; intra-assay CV, 6.1%; inter-assay CV, 6.4%) using a dedicated Architect device (ABBOTT Diagnostics Division, IL, USA, 840607/R4).

Statistical analysis

The statistical analyses were performed using the Statistica 13.3 package (StatSoft, Cracow, Poland). The variables were tested for a normal distribution using the Shapiro–Wilk test. Due to the non-normal distribution of the data, non-parametric tests were applied, and all values are expressed as the median and interquartile range. Categorical variables were compared via the χ^2 test. Continuous variables were compared among groups via one-way ANOVA. Spearman's test was used for correlation analysis. A P-value of <0.05 was considered statistically significant. The prevalence of comorbidities in the two DST groups was adjusted for sex with logistic regression.

Results

The clinical and biochemical characteristics of the studied groups are presented in Table 1. We examined 196 female and 99 male patients. In our study, 56 subjects (18.9%) had MACS (38 female and 18 male subjects) and the remaining 239 subjects (80.1%) had NFAT (158 female and 81 male subjects).

No difference in the frequency of obesity and hypertension was observed between patients with NFAT vs MACS ($P=0.9$, $P=0.2$; respectively) (Table 1) and no differences in the duration of hypertension ($P=0.08$) were noticed. Moreover, number of taken antihypertensive medicaments between patients with NFAT vs MACS did not differ (all $P>0.05$) (Table 2).

CKD was observed in 32.1% of MACS vs 28.5% of NFAT ($P=0.7$). We did not find differences in the diagnosis of prediabetes in MACS vs NFAT ($P=0.35$). Additionally, no differences in the frequency of impaired fasting glucose (16.1%) and impaired glucose tolerance (10.7%) in patients with MACS ($P=0.5$) were observed. Importantly, T2DM was diagnosed in 41% of MACS vs 23% of NFAT ($P<0.01$). Patients with MACS more frequently underwent one and two combined antidiabetic treatment interventions comparing to patients with NFAT (both $P<0.01$) (Table 2).

The higher frequency of hyperlipidaemia in NFAT than in MACS ($P=0.01$) has been noticed (Table 1). However, patients with MACS also more frequently underwent two combined hypolipemic therapies when comparing to NFAT ($P=0.03$), but higher number of patients with NFAT vs MACS subjects ($P<0.01$) were treated only with dietary interventions.

In the present study, we did not find differences in the prevalence of atrial fibrillation between studied

Table 1 Baseline characteristics of patients with NFAT and MACS. Values are expressed as median (interquartile range) and percent.

	NFAT (*n* = 239)	**MACS** (*n* = 56)	***P***
Age (years)	62 (54–68)	64 (58–71)	0.7
Gender (female/male)	158/81	38/18	–
BMI (kg/m^2)	30.5 (28–34)	31 (26–37.6)	0.6
Tumor size (mm)	18 (13–25)	26 (18–32)	<0.01
Bilateral (%)	19.2	39.3	0.01
ACTH (pg/mL)	19 (11–32)	13 (7–23)	0.01
1 mg DST (µg/dL)	1 (0.7–1.3)	2.3 (2–2.8)	<0.01
24 h UFC (nmol/L/24 h)	57 (30–105)	78 (39–121)	0.23
Cortisol saliva (nmol/L)	0.3 (0.1–0.6)	0.2 (0.1–0.8)	0.8
Total cholesterol (mg/dL)	192 (163–227)	184 (148–221)	0.1
LDL-cholesterol (mg/dL)	124 (93–157)	110 (78–153)	0.1
HDL-cholesterol (mg/dL)	54 (45–65)	52 (44–65)	0.4
Tg (mg/dL)	101 (83–137)	105 (75–140)	0.7
Glucose (mg/dL)	95 (89–105)	97 (92–114)	0.7
GFR (mL/min)	88 (76–99)	85 (75–102)	0.7
Hypertensive patient (%)	69.5	78.5	0.2
Prediabetes (%)	34.3	26.8	0.35
T2DM (%)	23	41	<0.01
Hyperlipidaemia (%)	72.4	53.6	0.01
Obesity (%)	34.7	33.9	0.9
CKD (%)	28.5	32.1	0.7
Stroke (%)	2.9	1.8	0.6
STEMI (%)	5	5.4	0.6
NSTEMI (%)	0.8	0	0.5
Coronary angioplasty (%)	5	5.4	0.6
Atrial fibrillation (%)	5.4	2.5	0.2

ACTH, adrenocorticotrophic hormone; CKD, chronic kidney disease; GFR, glomerular filtration rate; MACS, mild adrenal autonomous cortisol secretion, NFAT, non-functioning adrenal tumor; NSTEMI, non-ST elevation myocardial infarction; STEMI, ST elevation myocardial infarction; T2DM, type 2 diabetes mellitus; 1 mg-DST, serum cortisol levels after 1 mg dexamethasone suppression test; 24 h UFC, 24 h urinary free cortisol levels.

groups (*P*=0.2) and we did not observe differences in the cardiovascular events, such as stroke, ST and non-ST elevation myocardial infarction and coronary angiography between patients with NFAT and MACS (all *P*>0.05) (Table 1).

The patients with MACS were observed with higher frequency of bilateral lesion in comparison to patients with NFAT (*P*=0.01) (Table 1). In patients with MACS and bilateral lesion, the higher frequency of hyperlipidaemia (*P*=0.03), STEMI (*P*=0.02) and coronary angiography (*P*=0.01) in comparison to the patients with MACS and unilateral lesion was observed. In the group of patients with NFAT in bilateral vs unilateral lesion (*P*=0.04), the obesity was more frequent occurring.

Additionally, we assessed the incidence of suppression after the 1 mg dexamethasone suppression test in particular disease entities. In patients without obesity, we observed MACS in 19.2%, whereas in obese subjects, we observed MACS in 18.6% (*P*=0.9). In patients without hypertension, MACS was observed in 14.1%, whereas in subjects with hypertension, MACS was observed in 21% (*P*=0.2). In patients without prediabetes, MACS was

observed in 20.7%, whereas in subjects with prediabetes, MACS was observed in 15.5% (*P*=0.3). In patients without T2DM, MACS was observed in 15.2%, in comparison to 29.5% in patients with T2DM (*P*<0.01). In subjects without hyperlipidaemia, MACS was found in 28.3%, whereas in subjects with hyperlipidaemia, MACS was detected in 14.8% (*P*=0.01). In patients without CKD, MACS was observed in 18.2%, whereas in patients with CKD, MACS was diagnosed in 20.1% (*P*=0.7).

We did not observe differences in cortisol in midnight saliva and 24 h UFC between patients with and without MACS (all *P*>0.05); however, the plasma concentration of ACTH was lower in MACS than in NFAT (*P*=0.01) and tumour size was greater in MACS than in NFAT (*P*<0.01).

We did not find differences in 24 h UFC levels in obese and non-obese patients with NFAT (*P*=0.07) and MACS (*P*=0.9), in hypertensive and normotensive patients with NFAT (*P*=0.4) and MACS (*P*=0.2), in patients with and without prediabetes and T2DM with NFAT (*P*=0.9, *P*=0.2; respectively) and MACS (*P*=0.6, *P*=0.3; respectively). Furthermore, we did not notice differences in 24 h UFC levels in the group of NFAT (*P*=0.6) and MACS (*P*=0.8)

Table 2 Duration of hypertension and the number of antihypertensive, antidiabetic and hypolipidemic drugs in patients with NFAT and MACS. Data are present as a mean (minimum-maximum) and in percent.

	NFAT (*n* = 239)	MACS (*n* = 56)	*P*
Duration of hypertension (years)	3.6 (0–30)	5 (0–30)	0.08
One antihypertensive drug (%)	20.5	12.5	0.2
Two antihypertensive drugs (%)	21.3	23.1	0.8
Three or more antihypertensive drugs (%)	27.7	42.9	0.1
One antidiabetic drug (%)	16.7	21.3	<0.01
Two antidiabetic drugs (%)	5	16.1	<0.01
Three antidiabetic drugs (%)	1.3	3.6	0.2
One hypolipidemic drug (%)	46	41.1	0.6
Two hypolipidemic drug (%)	1.7	7.1	0.03
Without hypolipidemic drug, only diet (%)	24.7	5.4	<0.01

MACS, mild adrenal autonomous cortisol secretion; *n*, number of participants; NFAT, non-functioning adrenal tumour.

patients with and without hyperlipidaemia. Furthermore, the group of patients with and without CKD with NFAT (*P* = 0.1) and with MACS (*P* = 0.3) did not differ in 24 h UFC levels. Moreover, no differences in 24 h UFC levels in the group with and without cardiovascular events in patients with NFAT and MACS (all *P* > 0.05) were also noticed. However, we observed the positive correlation between 24 h UFC and fasting glucose in MACS (r = 0.37, *P* = 0.01) but not in NFAT (r = 0.04, *P* = 0.6) patients.

We observed a relationship between the size of the tumour and serum cortisol after 1 mg DXM in the whole group (r = 0.27, *P* < 0.01). However, we did not observe a relationship between tumour size and 24 h UFC or midnight saliva cortisol in MACS or NFAT (all *P* > 0.05). We found a negative relationship between the plasma level of ACTH and tumour size in MACS (r = −0.3, *P* = 0.03) and NFAT (r = −0.1, *P* = 0.04).

In our study, we did not find a relationship between BMI and midnight saliva cortisol, plasma ACTH, 24 h UFC in MACS or NFAT (all *P* > 0.05).

Additionally, no relationship between serum levels of cortisol after DST and blood pressure, fasting glucose and lipids in the patients with MACS and NFAT was noticed (all *P* > 0.05).

Discussion

In our study, we observed no differences in the presence of obesity, hypertension, CKD and PD between MACS

and NFAT patients. However, in the group of patients with MACS, we found a higher tendency to the presence of T2DM in comparison to those patients who showed suppression after 1 mg of dexamethasone. Interestingly, in the group with NFAT, hyperlipidaemia was observed more frequently than in the group with MACS.

One of the most important observations from this retrospective study is the fact that T2DM was more common in the group with MACS than in the group without MACS. Most studies have shown that even mild hypercortisolism is associated with varying degrees of impaired glucose metabolism with hyperinsulinemia (11). Therefore, the explanation of a higher tendency to T2DM in MACS could be connected as a causal link between hypercortisolism and insulin resistance (9). In previous studies, approximately one-third of MACS patients suffered from T2DM (11), whereas in our study, T2DM was diagnosed in 41% with MACS. It has been shown that the T2DM prevalence in MACS is largely variable among different studies due to the different procedures (i.e. fasting glucose vs oral glucose tolerance test) for T2DM diagnosis. In our study, in all patients who did not have previously recognised diabetes, an OGTT was performed. On the other hand, the MACS prevalence among patients with T2DM is difficult to pinpoint, due to the great heterogeneity among studies. We observed MACS in 15.2% of patients without T2DM, in comparison to 29.5% in subjects with T2DM. It should be mentioned that Delivanis *et al.* did not observe significant differences in T2DM or impaired glucose tolerance between patients with and without MACS (24). In our study, we did not find differences in PD between MACS and NFAT and we did not observe differences in the frequency of impaired fasting glucose and impaired glucose tolerance in patients with MACS. Interestingly, we observed a positive relationship between 24 h UFC and fasting glucose in patients with MACS. Accordingly, in a previously reported series of NFAT, T2DM was present in 21% of patients (25). This is consistent with our observation; we observed T2DM in 23% of subjects with NFAT. Similarly, in a study conducted by Fernandez-Real *et al.* (26), a higher prevalence of impaired glucose tolerance or unknown diabetes among patients with NFAT was presented. Diabetes was diagnosed in 22% and glucose intolerance was observed in 43% in this study. Additionally, the area under the curve of ACTH after CRH was not significantly suppressed in patients with disturbed glucose tolerance (26). Other authors presented areas under the curve for glucose and insulin during OGTT that were increased in NFAT (22). The mechanisms influencing the high prevalence of glucose intolerance in patients with NFAT were investigated, and it was reported

that cortisol production affects glucose metabolism or disturbed glucose tolerance (with its compensatory mechanisms) induces changes in adrenocortical function (26). It should be mentioned that in the last systemic meta-analysis and review, it was shown that T2D is present in 28.1% of patients with MACS and 14.4% of those with NFAT, while PD is present in 50% of MACS and 11.5% of NFAT (17).

In our study, we did not find differences in the diagnosis of hypertension between MACS and NFAT subjects (78.5% vs 69.5%). The obtained results indicate a relatively high percentage of patients with hypertension, both in the NFAT group and in the MACS group. In this case, this discrepancy could be connected to the characteristic of the studied population (Caucasian population). In a previous study, the observed prevalence of hypertension was 58% in patients with NFAT and 64% in subjects without suppression after 1 mg of dexamethasone (27). Other authors showed that blood pressure values were higher in adrenal incidentaloma than in the control group and 50% of patients with adrenal incidentaloma were hypertensive (22). In the last meta-analysis, hypertension was more frequently found in MACS than in NFAT (64.0% vs 58.2%) (17). Interestingly, Delivanis *et al.* did not observe significant differences in hypertension or T2D between patients with Cushing's syndrome, MACS and NFAT (24). Therefore, there is ongoing debate as to whether these diseases are associated with an increased risk of cardiovascular disease in MACS in comparison to NFAT. The mechanisms involved in the pathogenesis of cortisol-related hypertension are complex and still not fully clarified, especially in MACS. There are hypotheses that hypercortisolism is connected to increased activity of the renin–angiotensin–aldosterone system, an imbalance in the vasoregulatory system due to an inhibition of vasodilators and increased vascular reactivity to vasopressors, or the activation of renal mineralocorticoid receptor (11). It is important to underline that clear evidence of these mechanisms in MACS is still lacking. However, a loss in the physiological nocturnal blood pressure decrease has been observed (11). Additionally, we cannot exclude the influence of adrenal tumour size on the occurrence of comorbidities.

It has been shown that patients with MACS have an increased rate of dyslipidaemia, ranging from 12.4 to 78.8%. The pathogenesis of dyslipidaemia in hypercortisolism is complex; it is well established that cortisol affects lipolysis, hepatic very-low-density lipoprotein synthesis, fatty liver content and free fatty acid synthesis (28). This commonly results in raised total and LDL-cholesterol and triglyceride concentrations and reduced HDL-cholesterol

concentrations. Additionally, insulin resistance apparently plays a key role in the determination of the lipid pattern in MACS, as the absence of impaired glucose metabolism prevents the subtle cortisol excess from causing dyslipidaemia (11). Interestingly, in our study, we observed a higher frequency of occurrence of hyperlipidaemia in NFAT (72.4%) than in MACS (53.6%). Referring to the fact that we excluded from our study subjects with adrenal incidentaloma with different diseases, described in detail in the Materials and methods section. To the contrary, in the study conducted by Delivanis *et al.*, it was reported that patients with MACS had a higher prevalence of dyslipidaemia when compared to those with NFAT (67% vs 47%) (24). Interestingly, in the last systemic meta-analysis and review, it was shown that dyslipidaemia is present in 34.1% of patients with MACS and in 33.8% of those with NFAT (17). In the present study, we observed a higher incidence of dyslipidaemia both in patients with MACS and in those with NFAT in comparison to the above-cited studies.

Additionally, we did not observe differences in the prevalence of atrial fibrillation between the studied groups. However, in the study performed by Di Dalmazi *et al.*, the atrial fibrillation prevalence was higher in patients with MACS than NFAT and the general population (29).

In the present study, in the group with MACS, we observed obesity in 33.9%, whereas in NFAT, the prevalence of obesity was 34.7%. However, in a previous study, the prevalence of obesity was higher in MACS than in NFAT patients (11). Additionally, a significant increase in visceral fat content was found in patients with MACS after a follow-up period of 3 years, as compared with NFAT (30). Interestingly, in other studies, increased visceral fat mass was observed along with impaired glucose tolerance and hyperinsulinemia (22). Accordingly, in a recently published study, it was found that patients with NFAT have abnormal body composition, with increased subcutaneous and visceral fat and decreased muscle mass when compared to sex-, age- and BMI-matched referent subjects (24). In the last systemic meta-analysis and review, it was shown that obesity is present in 41% of patients with MACS and in 38.8% of those with NFAT (17).

In a retrospective study performed by Singh *et al.*, carried out on 168 patients with MACS and 275 patients with NFAT, it was found that the prevalence of CKD was significantly higher in patients with MACS than in those with NFAT. The authors hypothesised that this could be connected with the direct effect of cortisol on kidney function or due to a higher prevalence of hypertension in patients with MACS (31). In our study, CKD was observed

in 32.1% of MACS vs 28.5% of NFAT, whereas in the above-mentioned study, the authors observed that 25.3% of patients with MACS and 16.9% of patients with NFAT had CKD (31). In our study, in both groups, higher prevalence of CKD was observed in comparison to the above-cited study. This could be connected to the characteristics of the group, as we examined only Caucasian patients and would be worth exploring these characteristics in future perspectives studies. Accordingly, in most studies, CKD was not evaluated. From the other hand, the higher prevalence of diagnosis of hypertension in our cohort in comparison to the study performed by Singh *et al.* was noticed.

As mentioned in the Introduction section, MACS is observed in 15–50% of patients with adrenal incidentalomas (7, 8) and depends at first on the definition. In our study, we observed MACS in 18.9% of subjects, whereas 80.1% presented NFAT. A trend towards an increased frequency of MACS has been suggested. In earlier studies, MACS was diagnosed in 15–20% of patients, whereas the novel data recommended that, MACS has been observed in about 50% of cases (8).

Several studies observed the long-term outcomes in patients with MACS (7, 17, 32). Moreover, there is currently no level 3 evidence from large intervention randomised controlled trials to guide the management of MACS in adrenal incidentaloma. In a meta-analysis and review performed by Bancos *et al.*, it was found that adrenalectomy in patients with MACS is connected with 61% improvement in hypertension, 52% improvement in diabetes, 45% improvement in obesity and 24% improvement in dyslipidaemia. However, when compared with medical management of the cardiometabolic consequences of MACS, the patients who underwent adrenalectomy had a significant relative risk reduction in hypertension and diabetes but did not present a statistically significant improvement in dyslipidaemia or obesity (32). In other studies that assessed mortality in a total of 1356 patients with NFAT or MACS, during a mean follow-up period of 56.3 months, the overall proportion of reported deaths from any cause was relatively high (11.2% (CI, 9.5–13.0%)) and was similar in NFAT (12.0%) and MACS (11.5%) (17). In our study, we did not observe differences in the incidence of obesity, hypertension, PD, or kidney chronic disease in patients with NFAT and MACS. This might suggest that mild hypercortisolaemia is not associated with a higher cardiovascular risk in comparison to subjects with NFAT in our Caucasian population; however, prospective studies focusing on this topic are necessary, especially because these diseases are more commonly being observed. In the present study, no differences in the frequency of ST and non-ST elevation myocardial infarction, coronary angiography and stroke between NFAT and MACS have been noticed. Therefore, performing the prospective studies concerned on the surgery results assessment, in patients qualified accordingly to CV risk factors, are of great importance.

It has been shown that high 24 h UFC levels might be considered as a useful marker to identify the highest cardiovascular risk among patients who did not show suppression in the DST (21). However, in our study, we did not observe differences in 24 h UFC levels in the group with and without cardiovascular risk factors and events in patients with NFAT and MACS. It could be connected to the fact that we did not measure 24 h UFC levels with liquid Chromatography with tandem mass spectrometry but via the chemiluminescent microparticle immunoassay method.

The strengths of this study are the relatively large sample size and robust inclusion criteria. However, our research has several limitations. First, we collected clinical and biochemical data in a retrospective design, and we did not measure the dexamethasone level along with the morning cortisol to ensure that levels were therapeutic. Moreover, in our study, we did not evaluate cigarette smoking, which may play an influencing role. Furthermore, the study only included Caucasian individuals and thus may limit the generalisability of this study to other population groups for which different results could have been observed. Additionally, in the present study, the control group is lacking. It should be mentioned that the comparable control group to identify cardiovascular risk factors in MACS should be referred to patients without adrenal tumour. If such a control group was present then the study results could have been different. Accordingly, we also enrolled only patients hospitalised in the Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Białystok what would be also considered as limitation of this study. However, it should be emphasised that our department is the reference unit for this disease and most of the patients with adrenal incidentaloma from outpatients clinic are admitted to our department for diagnostic tests.

In conclusion, in our retrospective analyses, we found that T2DM is more prevalent in patients with MACS than in those with NFAT, whereas hyperlipidaemia is more prevalent in NFAT. Therefore, in the group with MACS, diabetes must always be ruled out, and an OGTT should be performed if diabetes is excluded on the basis of the fasting glucose serum level. Accordingly, in subjects with NFAT, the lipid profile must be tested, and a proper lifestyle

should be implemented. Additionally, no differences were observed in the incidence of obesity, hypertension, PD, or kidney chronic disease in patients with NFAT and MACS. This might suggest that mild hypercortisolaemia is not associated with higher cardiovascular risk in comparison to subjects with NFAT; however, further studies are warranted to confirm this. Future studies could explore non-Caucasian population groups as well as tumour size connected to the occurrences of co-morbidities.

References

1 Young WF. Clinical practice. The incidentally discovered adrenal mass. *New England Journal of Medicine* 2007 **356** 601–610. (https://doi.org/10.1056/NEJMcp065470)

2 Bednarczuk T, Bolanowski M, Sworczak K, Górnicka B, Cieszanowski A, Otto M, Ambroziak U, Pachucki J, Kubicka E, Babińska A, *et al.* Adrenal incidentaloma in adults – management recommendations by the polish society of endocrinology. *Endokrynologia Polska* 2016 **67** 234–258. (https://doi.org/10.5603/EP.a2016.0039)

3 Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, Tabarin A, Terzolo M, Tsagarakis S & Dekkers OM. Management of adrenal incidentalomas: European Society of Endocrinology clinical practice guideline in collaboration with the European network for the study of adrenal tumors. *European Journal of Endocrinology* 2016 **175** G1–G34. (https://doi.org/10.1530/EJE-16-0467)

4 Ceccato F, Barbot M, Scaroni C & Boscaro M. Frequently asked questions and answers (if any) in patients with adrenal incidentaloma. *Journal of Endocrinological Investigation* 2021 **44** 2749–2763. (https://doi.org/10.1007/s40618-021-01615-3)

5 Kloos RT, Gross MD, Francis IR, Korobkin M & Shapiro B. Incidentally discovered adrenal masses. *Endocrine Reviews* 1995 **16** 460–484. (https://doi.org/10.1210/edrv-16-4-460)

6 Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM & Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 1526–1540. (https://doi.org/10.1210/jc.2008-0125)

7 Terzolo M, Pia A & Reimondo G. Subclinical Cushing's syndrome: definition and management. *Clinical Endocrinology* 2012 **76** 12–18. (https://doi.org/10.1111/j.1365-2265.2011.04253.x)

8 Reimondo G, Castellano E, Grosso M, Priotto R, Puglisi S, Pia A, Pellegrino M, Borretta G & Terzolo M. Adrenal incidentalomas are tied to increased risk of diabetes: findings from a prospective study. *Journal of Clinical Endocrinology and Metabolism* 2020 **105** dgz284. (https://doi.org/10.1210/clinem/dgz284)

9 Di Dalmazi G, Pasquali R, Beuschlein F & Reincke M. Subclinical hypercortisolism: a state, a syndrome, or a disease? *European Journal of Endocrinology* 2015 **173** M61–M71. (https://doi.org/10.1530/EJE-15-0272)

10 Androulakis II, Kaltsas GA, Kollias GE, Markou AC, Gouli AK, Thomas DA, Alexandraki KI, Papamichael CM, Hadjidakis DJ & Piaditis GP. Patients with apparently nonfunctioning adrenal incidentalomas may be at increased cardiovascular risk due to excessive cortisol secretion. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 2754–2762. (https://doi.org/10.1210/jc.2013-4064)

11 Aresta C, Favero V, Morelli V, Giovanelli L, Parazzoli C, Falchetti A, Pugliese F, Gennari L, Vescini F, Salcuni A, *et al.* Cardiovascular complications of mild autonomous cortisol secretion. *Best Practice and Research: Clinical Endocrinology and Metabolism* 2021 **35** 101494. (https://doi.org/10.1016/j.beem.2021.101494)

12 Yozamp N & Vaidya A. Assessment of mild autonomous cortisol secretion among incidentally discovered adrenal masses. *Best Practice and Research: Clinical Endocrinology and Metabolism* 2021 **35** 101491. (https://doi.org/10.1016/j.beem.2021.101491)

13 Di Dalmazi G, Vicennati V, Garelli S, Casadio E, Rinaldi E, Giampalma E, Mosconi C, Golfieri R, Paccapelo A, Pagotto U, *et al.* Cardiovascular events and mortality in patients with adrenal incidentalomas that are either non-secreting or associated with intermediate phenotype or subclinical Cushing's syndrome: a 15-year retrospective study. *Lancet: Diabetes and Endocrinology* 2014 **2** 396–405. (https://doi.org/10.1016/S2213-8587(13)70211-0)

14 Di Dalmazi G, Vicennati V, Rinaldi E, Morselli-Labate AM, Giampalma E, Mosconi C, Pagotto U & Pasquali R. Progressively increased patterns of subclinical cortisol hypersecretion in adrenal incidentalomas differently predict major metabolic and cardiovascular outcomes: a large cross-sectional study. *European Journal of Endocrinology* 2012 **166** 669–677. (https://doi.org/10.1530/EJE-11-1039)

15 Sbardella E, Minnetti M, D'aluisio D, Rizza L, Di Giorgio MR, Vinci F, Pofi R, Giannetta E, Venneri MA, Vestri A, *et al.* Cardiovascular features of possible autonomous cortisol secretion in patients with adrenal incidentalomas. *European Journal of Endocrinology* 2018 **178** 501–511. (https://doi.org/10.1530/EJE-17-0986)

16 Podbregar A, Janez A, Goricar K & Jensterle M. The prevalence and characteristics of non-functioning and autonomous cortisol secreting adrenal incidentaloma after patients' stratification by body mass index and age. *BMC Endocrine Disorders* 2020 **20** 118. (https://doi.org/10.1186/s12902-020-00599-0)

17 Elhassan YS, Alahdab F, Prete A, Delivanis DA, Khanna A, Prokop L, Murad MH, O'reilly MW, Arlt W & Bancos I. Natural history of adrenal incidentalomas with and without mild autonomous cortisol excess: a systematic review and meta-analysis. *Annals of Internal Medicine* 2019 **171** 107–116. (https://doi.org/10.7326/M18-3630)

18 Terzolo M, Pia A, Alì A, Osella G, Reimondo G, Bovio S, Daffara F, Procopio M, Paccotti P, Borretta G, *et al.* Adrenal incidentaloma: a new cause of the metabolic syndrome? *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 998–1003. (https://doi.org/10.1210/jcem.87.3.8277)

19 Angeli A & Terzolo M. Adrenal incidentaloma – a modern disease with old complications. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 4869–4871. (https://doi.org/10.1210/jc.2002-021436)

20 Lopez D, Luque-Fernandez MA, Steele A, Adler GK, Turchin A & Vaidya A. 'Nonfunctional' adrenal tumors and the risk for incident diabetes and cardiovascular outcomes: a cohort study. *Annals of Internal Medicine* 2016 **165** 533–542. (https://doi.org/10.7326/M16-0547)

21 Ceccato F, Antonelli G, Frigo AC, Regazzo D, Plebani M, Boscaro M & Scaroni C. First-line screening tests for Cushing's syndrome in patients with adrenal incidentaloma: the role of urinary free cortisol measured by LC-MS/MS. *Journal of Endocrinological Investigation* 2017 **40** 753–760. (https://doi.org/10.1007/s40618-017-0644-8)

22 Garrapa GG, Pantanetti P, Arnaldi G, Mantero F & Faloia E. Body composition and metabolic features in women with adrenal incidentaloma or Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 5301–5306. (https://doi.org/10.1210/jcem.86.11.8059)

23 Levey AS, Eckardt KU, Dorman NM, Christiansen SL, Cheung M, Jadoul M & Winkelmayer WC. Nomenclature for kidney function and disease-executive summary and glossary from a kidney disease: improving global outcomes (kdigo) consensus conference. *European Heart Journal* 2020 **41** 4592–4598. (https://doi.org/10.1093/eurheartj/ehaa650)

24 Delivanis DA, Hurtado Andrade MD, Cortes T, Athimulam S, Khanna A, Atkinson E, Mckenzie T, Takahashi N, Moynagh MR & Bancos I. Abnormal body composition in patients with adrenal adenomas. *European Journal of Endocrinology* 2021 **185** 653–662. (https://doi.org/10.1530/EJE-21-0458)

25 Reincke M, Nieke J, Krestin GP, Saeger W, Allolio B & Winkelmann W. Preclinical Cushing's syndrome in adrenal 'incidentalomas': comparison with adrenal Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 1992 **75** 826–832. (https://doi.org/10.1210/jcem.75.3.1517373)

26 Fernández-Real JM, Engel WR, Simó R, Salinas I & Webb SM. Study of glucose tolerance in consecutive patients harbouring incidental adrenal tumours. Study group of incidental adrenal adenoma. *Clinical Endocrinology* 1998 **49** 53–61. (https://doi.org/10.1046/j.1365-2265.1998.00437.x)

27 Debono M, Bradburn M, Bull M, Harrison B, Ross RJ & Newell-Price J. Cortisol as a marker for increased mortality in patients with incidental adrenocortical adenomas. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 4462–4470. (https://doi.org/10.1210/jc.2014-3007)

28 Arnaldi G, Scandali VM, Trementino L, Cardinaletti M, Appolloni G & Boscaro M. Pathophysiology of dyslipidemia in Cushing's syndrome. *Neuroendocrinology* 2010 **92** (Supplement 1) 86–90. (https://doi.org/10.1159/000314213)

29 Di Dalmazi G, Vicennati V, Pizzi C, Mosconi C, Tucci L, Balacchi C, Cosentino ER, Paolisso P, Fanelli F, Gambineri A, *et al.* Prevalence and incidence of atrial fibrillation in a large cohort of adrenal incidentalomas: a long-term study. *Journal of Clinical Endocrinology and Metabolism* 2020 **105** dgaa270. (https://doi.org/10.1210/clinem/dgaa270)

30 Yener S, Baris M, Peker A, Demir O, Ozgen B & Secil M. Autonomous cortisol secretion in adrenal incidentalomas and increased visceral fat accumulation during follow-up. *Clinical Endocrinology* 2017 **87** 425–432. (https://doi.org/10.1111/cen.13408)

31 Singh S, Atkinson EJ, Achenbach SJ, Lebrasseur N & Bancos I. Frailty in patients with mild autonomous cortisol secretion is higher than in patients with nonfunctioning adrenal tumors. *Journal of Clinical Endocrinology and Metabolism* 2020 **105** e3307–e3315. (https://doi.org/10.1210/clinem/dgaa410)

32 Bancos I, Alahdab F, Crowley RK, Chortis V, Delivanis DA, Erickson D, Natt N, Terzolo M, Arlt W, Young WF, *et al.* Therapy of endocrine disease: improvement of cardiovascular risk factors after adrenalectomy in patients with adrenal tumors and subclinical Cushing's syndrome: a systematic review and meta-analysis. *European Journal of Endocrinology* 2016 **175** R283–R295. (https://doi.org/10.1530/EJE-16-0465)