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Clonidine suppression test for a reliable diagnosis of pheochromocytoma: When to use

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Abstract

Objective: In clinical practice, false-positive results in biochemical testing for suspected pheochromocytoma/paraganglioma (PPGL) are not infrequent and may lead to unnecessary examinations. We aimed to evaluate the role of the clonidine suppression test (CST) in the era of analyses of plasma-free metanephrines for the diagnosis or exclusion of PPGL in patients with adrenal tumours and/or arterial hypertension.

Design and Methods: This single-centre, prospective trial investigated the use of CST in 60 patients with suspected PPGL associated with out-patient elevations of plasma normetanephrine (NMN) and/or metanephrine (MN), in most cases accompanied with hypertension or an adrenal mass. Measurements of plasma catecholamines and free metanephrines were performed by liquid chromatography with electrochemical detection and tandem mass spectrometry, respectively.

Results: Forty-six patients entered final analysis ($n = 20$ with PPGL and $n = 26$ with a nonfunctional adrenal mass and/or hypertension). CST reliably excluded false-positive baseline NMN results with a specificity of 100%. The sensitivity of CST improved from 85% to 94% when tumours with isolated MN increase ($n = 3$) were not considered. In patients with elevated baseline NMN ($n = 24$), CST correctly identified all patients without PPGL. Patients with falsely elevated baseline NMN results ($n = 7$, 26.9%) exhibited increases of baseline NMN up to 1.7-fold above the upper reference limit.

Conclusion: CST qualifies as a useful diagnostic tool for differential diagnosis of borderline elevated plasma-free NMN in patients with suspected PPGL. In this context, CST helps to correctly identify all false-positive NMN screening results.

KEYWORDS

catecholamines, clonidine suppression test, metanephrine, normetanephrine, pheochromocytoma

1 | INTRODUCTION

Pheochromocytomas and paragangliomas, together referred to as PPGLs, are tumours that arise from chromaffin cells and represent very rare causes of endocrine hypertension (0.2%–0.6% of all hypertensive patients).[1,2] At least 15 PPGL-associated driver genes and 12 different genetic syndromes have been described to date. Germline mutations in subunits of succinate dehydrogenase (*SDHx*), *RET* proto-oncogene, von Hippel-Lindau (*VHL*), neurofibromatosis type 1 (*NF1*), transmembrane protein 127 (*TMEM127*), and MYC-associated factor X (*MAX*) genes account for up to 40% of PPGLs.[3] Patients with PPGL suffer from sustained or more often paroxysmal hypertension accompanied in many cases by a combination of hyperhidrosis, palpitations, tremor, nausea, and pallor. Undiagnosed PPGL might lead to a fatal outcome.[4]

Diagnostic tests for the presence of a PPGL are usually initiated on the grounds of signs and symptoms of catecholamine excess, clinical suspicion of secondary hypertension, an adrenal mass, previous history or family history of PPGL, or an established mutation in a tumour susceptibility gene. Several studies have demonstrated high sensitivity and specificity of the measurement of plasma-free metanephrine (MN) and normetanephrine (NMN).[5–7] Alternatively, fractionated metanephrines (in the following referred to MN and NMN together) can be measured in 24 h urine collections, but measurements of plasma-free metanephrines offer better diagnostic performance than the urinary panels, especially when it comes to patients at high risk for disease.[6–9] Liquid chromatography with mass spectrometric detection (LC-MS/MS) has been established as the method of choice for measurements of plasma-free metanephrines.[10–13]

Preanalytical factors that interfere with measurements of plasma-free metanephrines and thus may lead to false-positive results include medication, seasonal temperature variation,[14] activation of the sympathetic nervous system associated with stress (e.g., extreme illness), and acquiring blood samples in the upright position. De Jong et al. demonstrated that plasma NMN and MN levels in blood samples collected in the upright position were 30% and 12% higher than those collected after 30-min rest in the supine position.[15] In other large examined populations, supine, age-adjusted normetanephrine measurements were half as likely to be elevated than those acquired in the seated position.[16,17] Therefore, it is recommended to acquire blood samples after at least 20–30 min rest in a strictly supine position.[15–18] It is well known that the two medications that account for the majority of false-positive elevations of plasma or urinary noradrenaline and normetanephrine (up to 45%) are tricyclic antidepressants and phenoxybenzamine.[19] Other antihypertensive drugs like α_1-adrenoceptor blockers, calcium channel blockers, diuretics, and β-adrenoceptor blockers are not significant sources of false-positive results, and their withdrawal before testing is not necessary. Intake of β-adrenoceptor blockers may be associated with false-positive MN results in plasma. As the false-positive rate is not high (ca. 12.5%), routine withdrawal of this medication before testing for PPGL is not recommended, unless an equivocal result has been obtained and repeated testing seems reasonable.[19]

In clinical practice, false-positive results in biochemical testing for a PPGL are not infrequent.[20] In unselected patient populations, false-positive results for plasma-free or urinary fractionated metanephrines might occur in up to 22% of patients[21]; highest false-positive rates were observed for urinary metanephrines, especially urinary NMN.[20,21] In particular, while false positives in both MN and NMN are rare, isolated increases of NMN up to two- to threefold above the upper reference limit (URL) occur more often,[18–20] leading to repeated biochemical testing and imaging examinations until a pheochromocytoma is excluded or confirmed. Traditionally, the clonidine suppression test (CST) involving the measurement of catecholamines before and after oral clonidine administration was used to confirm or exclude the diagnosis of a PPGL. Since the implementation of routine measurement of MN and NMN, CST has become less frequent in clinical practice. In our study, we aimed to evaluate the utility of CST in the diagnostic algorithm for a PPGL, with emphasis on the catecholamine metabolites, and to identify the subpopulation of investigated patients who principally benefit from this test.

2 | MATERIAL AND METHODS

2.1 | Study population

A consecutive series of 60 patients, who underwent a CST in our department, a tertiary referral centre for endocrinology/endocrine tumours at the University Hospital Essen, Germany, between March 2013 and September 2017, were investigated in our study. All patients had clinical suspicion of PPGL and elevated plasma or urinary metanephrines or catecholamines in initial biochemical screening in our outpatient department or external clinics.

From the initially 60 patients who were evaluated for suspected PPGL, 14 were excluded from the final analysis because of incomplete data ($n = 3$, in one case because of a defect of the blood collection tube and in two cases because of insufficient labelling of the blood samples), metastatic pheochromocytoma ($n = 4$, metastases were diagnosed before CST was performed), use of tricyclic antidepressants ($n = 6$) or a central alpha-agonist ($n = 1$). A well-characterized effect of tricyclic antidepressants is blockade of noradrenaline reuptake into sympathetic nerve varicosities which results in increased noradrenaline spillover to plasma. Another key action of this medication class is central sympathoinhibition due to stimulation of α_2-adrenoceptors in the rostral ventrolateral medulla. The same mechanism of action accounts for the sympatholytic effect of clonidine.[22,23] Hence, no further decrease in sympathetic nerve discharge is expected in patients on tricyclic antidepressants, when clonidine is administered. For these reasons, the CST should not be performed in patients on tricyclic antidepressants or any other drug that blocks noradrenaline reuptake. There were no patients included in our study on phenoxybenzamine, sympathomimetics, MAO-inhibitors, α-Methyldopa, or any other substance with significant influence on plasma metanephrines.[10]

TABLE 1 Reasons to evaluate a catecholamine-producing tumour in the study population

	Number of patients ($n = 46$)
Hypertension, AM, and high (N)MN/CAT levels	19 (41%)
Hypertension and high (N)MN/CAT levels	11 (24%)
AM and high (N)MN/CAT levels	5 (11%)
Hypertension and AM	1 (2%)
Only AM	1 (2%)
Only high (N)MN/CAT levels	1 (2%)
PPGL associated gene mutation, hypertension, and AM	2 (4%)
PPGL associated gene mutation, AM, and high (N)MN/CAT levels	4 (9%)
History of PPGL and hypertension	2 (4%)

Note: Hormone measurements of the initial screening tests were obtained from plasma and/or 24 h urine samples and were analysed in different laboratories.

Abbreviations: AM, adrenal mass; CAT, catecholamines; MN, metanephrine; NMN, normetanephrine; PPGL, pheochromocytoma or paraganglioma.

The final study population consisted of 46 patients, 27 of whom were female. Of these, 35 patients had hypertension, 32 had an adrenal mass, and 27 complained about catecholamine-related symptoms. In seven patients a germline mutation was established (5 *RET*-, 1 *SDHB*-, and 1 *NF-1* mutation). Patient inclusion characteristics are summarized in Table 1.

Except for one new patient with *SDHB*-mutation, which was identified after inclusion in our study, all other mutation carriers were diagnosed before inclusion. Three of these patients were examined clinically and biochemically at regular intervals in our department because of already established MEN2a. These patients exhibited high plasma metanephrines and adrenal enlargement. The remaining three patients were presented to our clinic for the first time. One patient with *NF-1*- and one with *RET*-mutation were diagnosed externally, both harbouring an adrenal mass and elevated plasma metanephrines. The third patient had a family history of MEN2a and was recently diagnosed with thyroid and an adrenal tumour. The presence of the *RET*-mutation was confirmed shortly before inclusion in our study. The patient with newly diagnosed *SDHB*-mutation after inclusion, was a 21-year-old woman with resistant hypertension, adrenal mass, and elevated plasma metanephrines externally. Five of these seven patients with germline mutations in susceptibility genes complained about catecholamine-related symptoms.

Data of the initial biochemical screening were available for 42 of 46 patients. Initial screening for PPGL included measurements of metanephrines in plasma ($n = 37$) and urine ($n = 6$) as well as measurements of plasma ($n = 2$) and urinary catecholamines ($n = 10$). When both metanephrines and catecholamines were available ($n = 8$), metanephrines were considered for further investigations. In two patients, no measurement of plasma or urine metanephrines had been obtained; hence, urinary catecholamines were used as the initial screening test (Table 2). The used analytical technique was LCMS/MS in 36 patients, radioimmunoassay (RIA) in three patients, and high-pressure liquid chromatography (HPLC) in four patients (1 patient had 2 measures, one with LCMS/MS and one with HPLC with concordant result).

TABLE 2 Results of the initial screening before clonidine suppression test

Result of initial screening	Number of patients ($n = 42$) (PPGL/non-PPGL)
Negative screening	1 (0/1)
Only plasma MN increased	6 (6/0)
Only plasma NMN increased	21 (5/16)
Both plasma MN and NMN increased	9 (7/2)
Urinary NMN ± MN increased	3 (1/2)
Urinary catecholamines increased	2 (0/2)

Note: As increased was defined a value above the upper reference level.

Abbreviations: MN, metanephrine; NMN, normetanephrine; PPGL, pheochromocytoma/paraganglioma.

Twenty patients were diagnosed with a pheochromocytoma or paraganglioma (PPGL group), whereas a PPGL was excluded in 26 patients (non-PPGL group). Diagnosis of PPGL was confirmed histologically, except for two patients with a genetic mutation in the *RET* proto-oncogene. In both cases, patients had mild clinical features of catecholamine excess and refused surgical removal of the tumour. Diagnosis of PPGL in these two patients was made due to detection of a PPGL-typical adrenal mass in DOTATOC-PET/CT and MRI as well as elevated plasma metanephrines. Nineteen PPGLs were localized in the adrenal gland and one paraganglioma was located in the left pararenal region. Other causes of hormonally active adrenal tumours (Conn's syndrome, Cushing's syndrome, and androgen-producing tumours) as well as a congenital adrenal hyperplasia were excluded by endocrine testing in all patients with an adrenal mass.

A PPGL was excluded by at least one of the following criteria: normal baseline (0 h) MN and NMN concentrations before clonidine administration, which served as a repeated biochemical examination under optimal conditions, a negative follow-up with normal plasma MN and NMN for up to 2 years, a negative adrenal imaging and in some cases additional functional imaging and histological examination of the adrenal tumour. Patient characteristics are summarized in Table 3.

The study protocol was approved by the ethics committee of the University Hospital Essen. Written consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used.

2.2 | Clonidine suppression test

The CST was performed in hospitalized patients between 8:00 AM and 9:00 AM in the morning. Patients were admitted to the clinic the day before the CST was scheduled and an intravenous forearm

TABLE 3 Patient characteristics and plasma metanephrine results during clonidine suppression test

	PPGL total (n = 20)	PPGL sporadic (n = 13)	PPGL hereditary (n = 7)	Non-PPGL (n = 26)
Age (years)	48.5 (21–77)	52 (30–77)	33 (21–68)	58 (28–79)
Sex (f/m)	10/10	6/7	4/3	17/9
BMI (kg/m^2)	24.3 (19.1–34.7)	24.4 (19.1–31.2)	24.2 (20.4–34.7)	26.7 (13.5–58.1)
Blood pressure syst./diast. (mmHg)	130.5 (90–191)/71 (57–97)	108 (90–151)/68 (57–97)	136 (97-191)/73 (62–82)	139 (97–176)/70.5 (55–109)
NMN 0 h (pg/ml)	371.7 (45.7–6844.4)	1051.1 (45.7–6844.4)	172.7 (91.1–847.8)	117.2 (40.4–312.2)
NMN 3 h (pg/ml)	377.3 (33.7–6800)	1018.9 (33.7–6800)	155.6 (77.2–877.8)	70.3 (13–175.6)

Note: The results refer to medians (range). Sex distribution is presented in absolute numbers. To convert pg/ml to nmol/l, divide by 197 for metanephrine and 183 for normetanephrine.

Abbreviations: BMI, body mass index; MN, metanephrine; NMN, normetanephrine; PPGL, pheochromocytoma/paraganglioma.

cannula was inserted. They were instructed to avoid interfering medication, smoking, and food intake for 12 h before the test. During the test, patients remained supine and blood pressure and heart rate were monitored every 30 min. Blood samples were drawn from the indwelling intravenous cannula after at least 30 min of supine rest and 3 h after oral ingestion of clonidine. The dose of clonidine was adjusted to body weight: 300 µg for patients with body weight between 60 and 80 kg and 4.2 µg/kg for patients outside that range. Blood samples were collected into 10-ml heparinized tubes were immediately placed in prepared cooled boxes. Plasma was separated and stored frozen at −18°C. The samples were transported on dry ice to the Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Dresden, for further analysis. A CST result was considered to be normal if NMN was reduced to below the age-adjusted URL 3 h after clonidine administration or decreased at least 40% compared with baseline.[10,19] The lowest and highest age-adjusted URL for NMN used in our study population was 112 pg/ml (0.61 nmol/l) and 190 pg/ml (1.04 nmol/l), respectively.

2.3 | Analyses of plasma catecholamines and metanephrines

Measurements of plasma concentrations of catecholamines and free metanephrines were performed using liquid chromatography with electrochemical detection (HPLC-ECD) and tandem mass spectrometry (LC-MS/MS) respectively.[24,25] The diagnostic upper cutoff for MN was 84 pg/ml (0.43 nmol/l), whereas age-specific upper cutoffs were used for NMN.[26] URL was 84 pg/ml (0.46 nmol/l) for adrenaline and 497 pg/ml (2.94 nmol/l) for noradrenaline.

2.4 | Statistical analysis

Statistical analyses were carried out using IBM SPSS STATISTICS Version 27 and R Version 3.4.3. software. The results were expressed as median and range and were analysed with descriptive statistics. Comparisons of nonparametric continuous parameters were performed with Mann–Whitney U test. A value of $p < .05$ was considered statistically significant. For the presentation of MN and NMN values during CST (0 and 3 h) in Figures 1 and 2 a logarithmic transformation of the data (log10) was used.

3 | RESULTS

3.1 | Patients characteristics

Patient age ranged between 21 and 79 years, with a median age of 48.5 years in the PPGL group and 58.0 years in the non-PPGL group (p = .037). A lower body mass index (BMI) and a lower blood pressure (BP) were observed in the PPGL-group than in the control group at baseline (0 h) of CST, but these differences were not statistically significant (BMI p = .458; BPsyst. p = .078, BPdiast. p = .641) (Table 3). A significant difference was observed in the number of antihypertensive medications between the PPGL and non-PPGL group (PPGL: median 0 drugs/person [0–5], non-PPGL: median 2 drugs/person [0–5], p = .015).

3.2 | Initial screening and baseline metanephrines (0 h) of CST

Results of the initial screening parameters, before performing the CST, were available for 42 patients, 19/20 of the PPGL group and 23/26 of the non-PPGL group. All patients with PPGL and 22 of 23 patients (95.7%) without PPGL had pathological initial screening (elevated NMN, MN, or both). Baseline MN and NMN (0 h) of the CST, before clonidine administration, served as a second measurement under optimal conditions. In 21 cases the same catecholamine metabolite was elevated, both in the initial screening and at baseline of the CST. In the remaining 21 cases (50%) at least one of the two catecholamine metabolites was normal in one measurement and elevated in the other (Table 4).

The sensitivity of baseline catecholamines (0 h) of the CST for the diagnosis of a PPGL was 78.9% and specificity 39.1%. Baseline

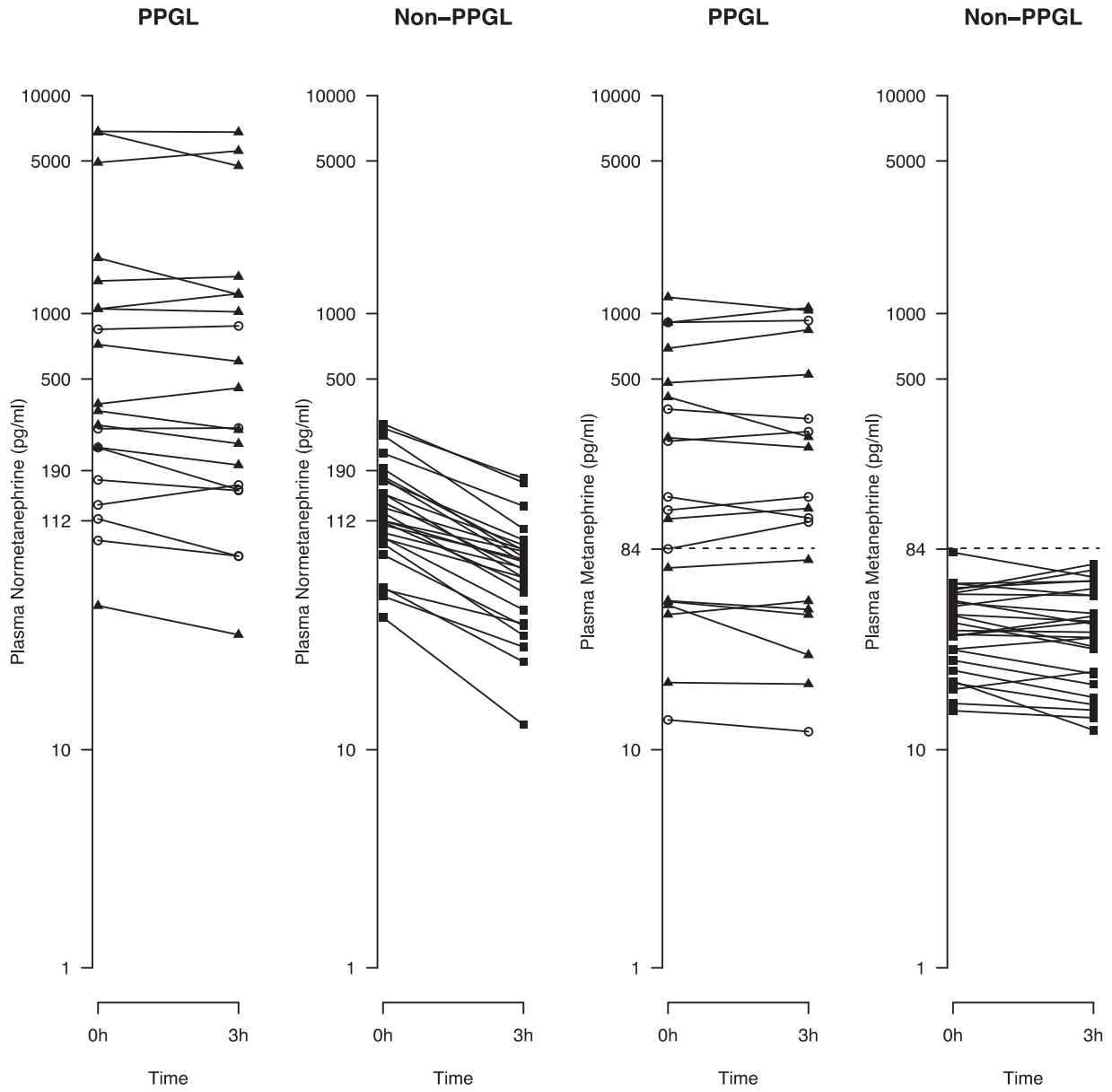

FIGURE 1 Plasma normetanephrine and metanephrine during clonidine suppression test in pheochromocytoma/paraganglioma (PPGL) and non-PPGL patients. Data of normetanephrine (NMN) and metanephrine (MN) are demonstrated after logarithmic transformation (log10). The dashed horizontal lines represent the upper reference limit (URL) of plasma metanephrine (84 pg/ml). The age-specific URL of normetanephrine ranged in our study population between 112 pg/ml and 190 pg/ml. The filled squares represent patients without pheochromocytoma, the empty circles patients with hereditary pheochromocytoma, and the filled triangles patients with sporadic pheochromocytoma.

metanephrines (0 h) before clonidine intake exhibited a sensitivity of 100% and a specificity of 73% (Table 5). The false-positive rate of 26.9% was attributed to seven subjects of the non-PPGL group with falsely elevated NMN at baseline (0 h). In these seven cases, we identified in the clinical patient history following conditions that might correlate with the elevated baseline NMN: one patient suffered from metastatic medullary thyroid carcinoma with B-symptoms and chronic pain due to skeletal metastases; one patient suffered from severe panic attacks; one patient reported chronic pain, sleep disorders and depression and one patient suffered from fibromyalgia, chronic diffuse arthralgia, and depression. The last two patients were also on Venlafaxine. One patient suffered from severe hypertension with recurrent hypertensive episodes up to 280 mmHg systolic and in two cases no specific reasons could be identified in the clinical and medical history of the patients.

3.3 | Results of clonidine suppression test

Analysis of 3 h-noradrenaline concentrations of the CST provided low sensitivity for the diagnosis of a PPGL (36.8% if the test was considered to be negative when 3h-values normalized and 26.3% if

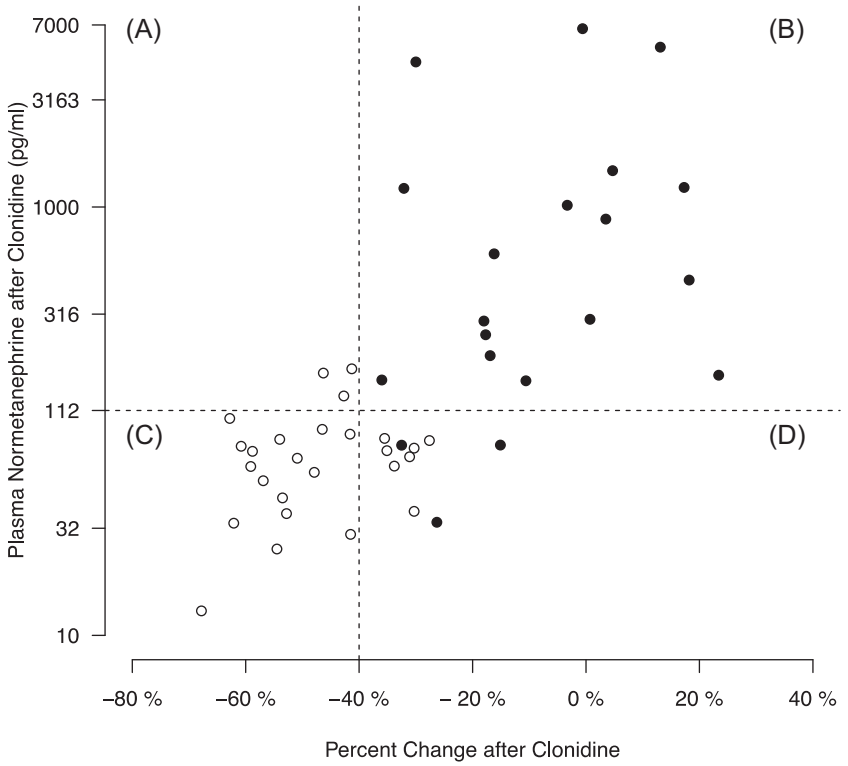

FIGURE 2 Normetanephrine results at the end of the clonidine suppression test. Data of normetanephrine (NMN) are demonstrated after logarithmic transformation (log10). The dashed horizontal line represents the lower upper reference limit of plasma normetanephrine at the end of the clonidine suppression test (CST) (112 pg/ml) and the dashed vertical line the 40% decrease of NMN 3 h after clonidine. The filled circles represent patients with and the empty circles patients without pheochromocytoma (PPGL). The distribution of patients without PPGL in areas (A), (C), and (D) shows the high specificity of the CST (100%), with no false-positive results. The three patients with PPGL in the area (D) represent patients with isolated metanephrine (MN) elevations, who had also normal NMN at the beginning of the CST (false negatives).

TABLE 4 Discrepancies in biochemical results between initial screening and baseline (0 h) of clonidine suppression test (CST) in 21 patients

	Initial screening result	Baseline of CST	Number of patients (n = 21)
PPGL			
	Only MN elevated	Only NMN elevated	1
	MN and NMN elevated	Only NMN elevated	2
	Only MN elevated	MN and NMN elevated	2
Non-PPGL			
	Only NMN elevated	MN and NMN normal	12
	MN and NMN elevated	MN and NMN normal	1
	MN and NMN elevated	Only NMN elevated	1
	CAT elevated	MN and NMN normal	2

Abbreviations: CAT, catecholamines; MN, metanephrine; NMN, normetanephrine; PPGL, pheochromocytoma or paraganglioma.

the test was considered to be negative when 3 h-values decreased >50% compared with baseline but remained above the URL). In all patients without PPGL noradrenaline normalized at the end of CST (specificity 100%).

With regard to 3 h-NMN results, CST was pathological in 17 of 20 patients with PPGL and in none of 26 patients without PPGL. The sensitivity of CST in our cohort was 85%, but specificity reached 100% (Table 5, Figure 1). Maximum NMN decreases 3 h after clonidine administration in the PPGL group was 32.5%. CST exhibited a rate of false-negative results of 15% (3/20 patients). This was attributed to three patients with isolated elevations of plasma MN (Figure 2).

The subgroup of patients with elevated baseline (0 h) NMN during CST (n = 24) comprised 17 patients of the PPGL group and 7 patients of the non-PPGL group. NMN remained elevated (above the age-specific URL) in all patients of the PPGL group (sensitivity 100%) and normalized in all seven patients of the non-PPGL group (specificity 100%) at the end of test. Thus, only 17 of 24 (70.8%) patients with elevated baseline (0 h) NMN had a PPGL. All patients of this subgroup without PPGL (n = 7) presented with slightly increased baseline NMN (0 h of the CST) between 1.02- and 1.64-fold above the URL. However, up to a 1.7-fold increase of NMN at baseline was also found in almost one-quarter (4/17, 23.5%) of noradrenergic PPGLs; in 23.5% an increase between 1.7- and 3-fold was

TABLE 5 Diagnostic performance of baseline (0 h) plasma catecholamines and metanephrines and outcome of clonidine suppression test

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Adrenaline[a]	42.1	81	66.7	60.7
Noradrenaline[a]	57.9	45.8	45.8	57.9
Adrenaline and Noradrenaline together[a,b]	78.9	39.1	51.7	69.2
Metanephrine[a]	60	100	100	76.5
Normetanephrine[a]	85	73.1	70.8	86.4
Metanephrine and Normetanephrine together[a,b]	100	73	74	100
Clonidine test result	85	100	100	89.7
(3 h-NMN levels)	**94.1**	**100**	**100**	**96.3**

Note: Results in bold refer to measures after exclusion of PPGLs with isolated MN increase ($n = 3$).

Abbreviations: NMN, normetanephrine; NPV, negative predictive value; PPV, positive predictive value; URL, upper reference limit.

[a]These results refer to baseline measures.

[b]These results refer to diagnostic performance when at least one of the mentioned parameters was elevated above the specific URL.

documented and the remaining 53% of PPGLs presented with more than threefold elevated baseline NMN levels (Figure 1).

In contrast, all patients with elevated baseline MN had a pheochromocytoma, and MN levels at the end of CST were not influenced by clonidine administration.

3.4 | Plasma metanephrines at baseline (0 h) and at the end of CST after exclusion of PPGLs with isolated MN increases

We assumed that PPGLs that present with an isolated plasma-free MN increase pose the main cause of reduced sensitivity of the CST.[27,28] To verify this assumption we analysed the data of the PPGL group after exclusion of patients with isolated MN increases ($n = 3$, two patients with hereditary RET-associated PPGL and one with sporadic PPGL). The combination of baseline (0 h) plasma metanephrines still showed a high diagnostic sensitivity of 100% and a specificity of 73.1%. The sensitivity of the CST result (3 h) improved from 85% to 94.1% and negative predictive value (NPV) from 89.7% to 96.3% with equivalent high specificity of 100% as in the entire study population (Table 5). There was still one patient in the PPGL-group with a negative CST result (initially elevated NMN that normalized after 3 h) and concomitant high MN associated with a RET mutation. Despite negative CST result, the diagnosis of PPGL would not have been missed in this patient, because of elevated MN.

4 | DISCUSSION

This prospective study successfully confirmed the utility of CST in the diagnostic procedure for a catecholamine-producing tumour. With a very high specificity of 100% CST correctly identifies patients with falsely elevated plasma NMN results before clonidine. The target group for this test is patients with mildly elevated NMN < 1.7-fold of the URL at a screening in an ideal setting.

As clonidine administration does not have a notable influence on adrenaline metabolism to metanephrine in the adrenal medulla, CST is not recommended in cases of isolated elevated MN.[19,29] Consistent with this, there was no relevant decrease of elevated MN levels at the end of CST in our cohort. In contrast, plasma NMN derives mainly from noradrenaline released from the sympathetic nerve endings as a result of sympathetic nerve activation and is, therefore, more susceptible to be false positive. Consequently, the specificity of this biochemical test can be improved by optimizing all potential confounders that activate the sympathetic nervous system before blood sampling (e.g., physical activity, smoking, medication, and seated position). Numerous studies demonstrated lower rates of false-positive results, when blood sampling is performed after prolonged supine rest.[15–17,20] Moreover, there is evidence that stress caused by direct venipuncture results in higher MN, NMN, and adrenaline levels compared with those acquired using an indwelling intravenous cannula.[30]

Although we attempted to minimize all these interfering factors in our study, we still observed a false-positive rate of 26.9% (7/26) for baseline plasma NMN (0 h) of CST. The impact of physical stress due to chronic pain syndromes and emotional/psychological disorders like panic attacks, depression, and anxiety as well as other comorbidities (e.g., symptomatic heart or renal disease) may be often underestimated in clinical routine. We could identify at least one of the above-mentioned factors in five of seven patients with false-positive NMN increases at baseline of CST. Moreover, two of these patients were on venlafaxine, a serotonin-norepinephrine reuptake inhibitor. Indeed, previous case reports provided evidence that venlafaxine can lead to remarkably falsely elevated NMN levels >4-fold of the URL,[31,32] because of its inhibitory action on noradrenaline reuptake in the sympathetic varicosities.[33]

All of the above-mentioned troublesome causes for false-positive results seem to have an even greater impact on biochemical screening for PPGL by primary and secondary healthcare providers and account for fluctuation of these parameters in repeated testing. The analysis of the initial biochemical screening that lead to further investigation for the presence of a PPGL and inclusion in our study, revealed a high prevalence of inconsistent results when compared with baseline (0 h) plasma metanephrines of CST. In only half of the cases, both MN and NMN were normal or elevated in both examinations (Table 4). Of note, not all laboratories use age-adjusted reference levels for NMN,[26] so this could account for at least a part of the observed discrepancies.

This observation underlines the importance of repeated biochemical testing under optimal conditions before proceeding to further imaging examinations, especially when screening applies to low-risk populations and in case of slight elevations of plasma metanephrines up to twofold above the URL. When drug interference is suspected, the possibility of drug discontinuation or switch to alternative drugs should be discussed with the treating physician. If this is not feasible and plasma metanephrines are persistently elevated follow-up should be carefully weighed against imaging tests (Figure 3).

In our cohort, we identified two patients with noradrenaline decrease of >50% but lack of normalization at the end of CST, who nonetheless had a pheochromocytoma and pathological 3 h NMN values. Hence, the cutoff of 50% for noradrenaline decrease in CST proposed in the literature[19,34] was not reproducible in our study population. Other studies in a time when only catecholamines were measured routinely during CST showed variable results (sensitivity between 20% and 97% and specificity between 32% and 93%), which did not allow a reliable confirmation or exclusion of a PPGL.[35–38] Diagnostic inaccuracy relates to catecholamine metabolism. Catecholamine secretion from chromaffin cells and PPGLs occurs actively via exocytosis in a variable and episodic manner. Independent of their secretion, cetecholamines also leak continuously from storage vesicles into the cytoplasm of chromaffin cells, where they

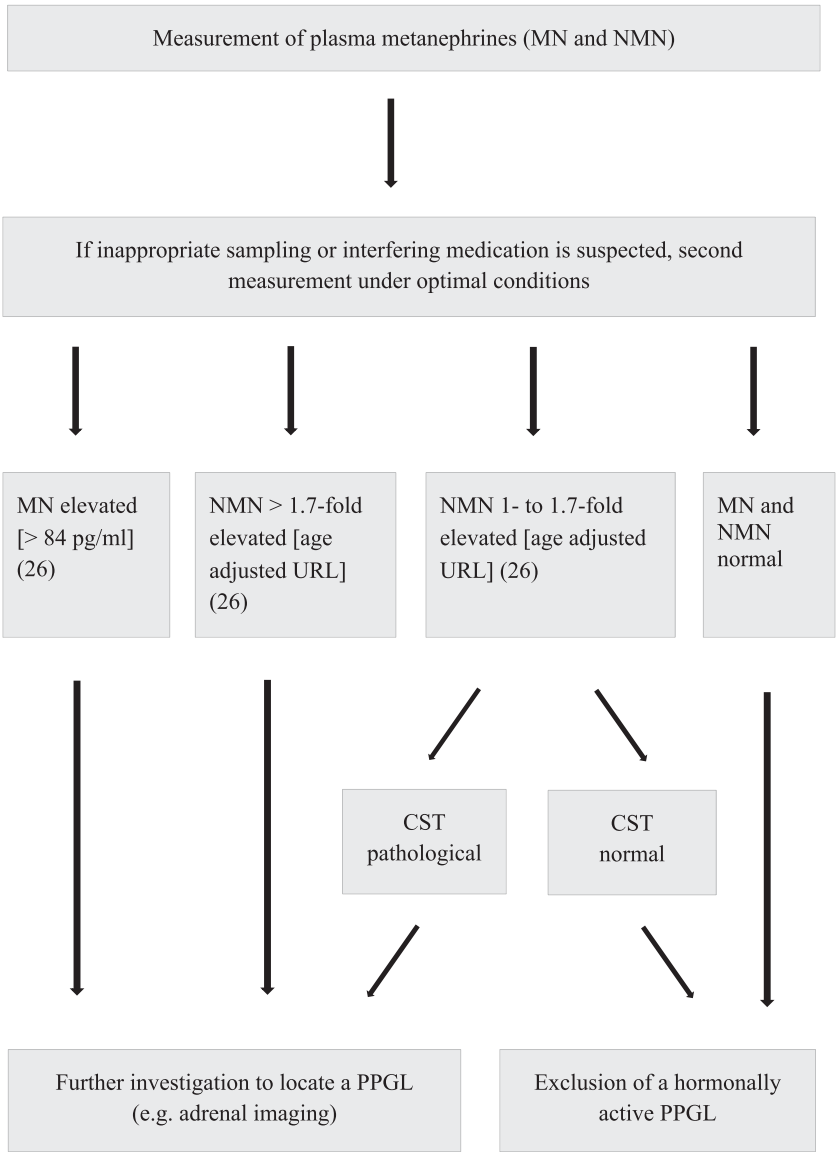

FIGURE 3 Proposed diagnostic algorithm for the diagnosis of a secretory PPGL. CST, clonidine suppression test; MN, metanephrine; NMN, normetanephrine; PPGL, pheochromocytoma or paraganglioma; URL, upper reference limit

metabolize to metanephrines in the presence of catechol-O-methyltransferase (COMT). Then, metanephrines diffuse passively from chromaffin cells into circulation.[39] Therefore, catecholamine measures are not recommended in the diagnostic procedure for a PPGL neither as a screening tool nor as part of the CST.

In summary, in our prospective study, we verified the utility of CST for the diagnosis of PPGL. Because of the improved chromatographic assays when compared to immunoassays[11,12] and the superior diagnostic performance that provide the more precise upper reference levels for metanephrines established in the past few years,[9,18,26] the majority of patients with PPGL can be correctly identified by measurements of MN and NMN in plasma and urine. This is particularly the case when patients at high risk for disease are tested, for example, due to clinical symptoms of catecholamine excess or a mutation in a susceptibility gene for PPGL as well as past or family history of PPGL.[40] In the most common clinical scenario, where patients at low risk for disease are tested and slight increases of NMN are not infrequent, a reliable confirmatory test with high specificity is needed. In our study, CST demonstrated diagnostic benefit in patients with slightly elevated plasma NMN between 1- and 1.7-fold above the age-adjusted URL, which accounted for approximately one-quarter of the screened patients (11/46, 23.9%). For these patients, CST proved to be a reliable confirmatory test, which excluded all healthy individuals (specificity 100%). Hence, we propose a diagnostic algorithm as demonstrated in Figure 3.

This study has a few limitations. The main limitation is the small patient number and the single-centre design. Whether our results can be extrapolated to the general population remains to be established. Another limiting factor is the heterogeneity of the initial screening for PPGL, being performed unstandardized in different settings and laboratories, not always using the same analytical method as in the CST (LCMS/MS). However, this was part of our study design, which aimed to reflect real-world data, as they occur in clinical routine, to uncover the weaknesses of the usual screening procedures for PPGL. To our knowledge, this is the first prospective study evaluating CST with measurements of NMN using LCMS/MS and well-established, age-specific reference levels[26] as well as comparing screening results with baseline results (0 h) of the CST.

In conclusion, measurements of plasma-free metanephrines under optimal pre- and analytical conditions can minimize the rate of false-positive results. In case of persistent borderline elevations of NMN, despite optimal sampling conditions, CST qualifies as a useful diagnostic tool to reliably exclude a PPGL.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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