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Evaluation of Endocrine Tests. C: glucagon and clonidine test in phaeochromocytoma

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ABSTRACT

Background: The diagnosis of phaeochromocytoma is based on the demonstration of catecholamine excess. Urine and plasma metanephrine measurements are highly sensitive tests for the diagnosis of phaeochromocytoma, but moderate elevations in metanephrines lack optimal specificity.

In this study we aimed to evaluate the diagnostic value of additional tests, i.e. glucagon stimulation and clonidine suppression test, in patients with moderately elevated catecholamines and/or metanephrines.

Methods: Patients with suspected phaeochromocytoma with moderately elevated catecholamines and/or metanephrines in plasma or urine were subjected to the glucagon stimulation and clonidine suppression test. The presence of phaeochromocytoma was confirmed by histology and the absence by a disease-free extended follow-up.

Results: Fifty-five patients were included. Phaeochromocytoma was diagnosed in 11 patients. The follow-up period in patients without phaeochromocytoma was 56 (19 to 154) months. The sensitivity of the glucagon test was 30% and the specificity 100%. The clonidine test had no discriminative power, because the area under the ROC curve was not significantly different from 0.5.

Conclusion: The clonidine suppression test without normetanephrine measurements and the glucagon stimulation test are not sensitive enough to safely exclude phaeochromocytoma in patients with mildly elevated plasma or urine catecholamines.

KEYWORDS

Clonidine, glucagons, phaeochromocytoma

INTRODUCTION

The early diagnosis of phaeochromocytoma is important, because unrecognised phaeochromocytoma is a potentially lethal condition. The diagnosis, however, poses a challenge for every physician. A relatively large number of patients may present with only minor signs and symptoms. In a Swedish study, the diagnosis was made only at autopsy in 40% of 439 patients with phaeochromocytoma, whereas phaeochromocytoma was an incidental finding in 14%.[1] On the other hand, in a series of patients clinically suspected of phaeochromocytoma (on the basis of signs and symptoms), the diagnosis was established in only one of 300.[2] Of patients with hypertension, phaeochromocytoma may be found in only ~0.1%.[3] Since missing the diagnosis could have serious consequences, diagnostic testing demands a high degree of sensitivity. In daily practice, biochemical testing aimed at the demonstration of excessive catecholamine production is performed. Biochemical tests include plasma epinephrine, norepinephrine and/or metanephrines, and 24-hour urinary excretion of epinephrine, norepinephrine and their O-methylated metabolites metanephrine and normetanephrine.[4] The demonstration of increased levels of plasma or urinary catecholamines and their metabolites should suffice to make a diagnosis of phaeochromocytoma likely. However, mildly elevated concentrations of catecholamines and their metabolites may be aspecific and could provide a dilemma as to further management given the low prevalence of phaeochromocytoma. To address this issue we prospectively analysed the value of additional dynamic tests for phaeochromocytoma in patients showing a mild catecholamine excess at initial screening. Either a provocative test with intravenous glucagon and/or a suppressive test with oral clonidine can be performed.

The current literature is not conclusive about the relative merits of these dynamic tests due to small patient series, differently defined control groups and differences in analytical assays.[3,5,6] Reported sensitivities and specificities for the glucagon provocation test were 60 to 81% and 100%, respectively, and for the clonidine suppression test 97% and 67 to 99%, respectively.[3,5,6] Grossman *et al.* reported a sensitivity of 100% and a specificity of 79% for the combination of the glucagon and clonidine test.[5] The present study was designed to prospectively evaluate the diagnostic accuracy of the glucagon provocation test and the clonidine suppression test for diagnosing phaeochromocytoma in groups of patients frequently encountered in an outpatient clinic of Internal Medicine/ Endocrinology, namely patients with clinical suspicion of phaeochromocytoma, an adrenal incidentaloma or genetic predisposition to phaeochromocytoma combined with a positive initial biochemical screening.

MATERIALS AND METHODS

Subjects

Since 1993 biochemical testing for phaeochromocytoma at the Academic Medical Center (University of Amsterdam) has been carried out by a stepwise approach. Initial screening consists of measurement of plasma epinephrine and norepinephrine, combined with measurement of 24-hour urinary excretion of epinephrine and norepinephrine and their metabolites metanephrine and normetanephrine. Fasting plasma catecholamines were collected from an indwelling venous catheter 30 and 45 minutes after insertion of the venous catheter while patients were in a supine position. Two consecutive 24-hour urine samples were collected while patients refrained from coffee, nuts, bananas and alcohol. If any of the measured concentrations were above the institutional reference value (plasma: epinephrine >0.55 nmol/l, norepinephrine >3.25 nmol/l; urine: epinephrine >275 nmol/24 hours, norepinephrine >890 nmol/24 hours, metanephrine>0.80 µmol/24 hours, normetanephrine>2.00 µmol/24 hours) an additional glucagon stimulation and clonidine suppression test were performed. Only in patients with plasma catecholamine concentrations exceeding 11.1 nmol/l were the glucagon and clonidine tests skipped and imaging of the adrenals was performed.[7] For this study we included all patients between 1993 and 2005 with a positive initial screening who underwent subsequent glucagon and clonidine testing. These patients had been screened for phaeochromocytoma because of 1) symptoms and signs that could fit the diagnosis phaeochromocytoma 2) an adrenal incidentaloma, 3) genetic predisposition for phaeochromocytoma (multiple endocrine neoplasia type 2 or succinate dehydrogenase complex subunit D mutation. 4) a history of paraganglioma/phaeochromocytoma. Exclusion criteria were parental drug abuse, alcohol abuse and pregnancy. Antihypertensive drugs or any other medication interfering with the tests was stopped or switched to doxazosin at least five days, but preferably two weeks, before the tests. Additional exclusion criteria were RR >160/100 mmHg to carry out the glucagon stimulation test and RR <100/60 mmHg to carry out the clonidine suppression test. In case of a positive glucagon and/or clonidine test a CT scan or MRI was performed, followed by MIBG scanning when indicated. Each diagnosis of phaeochromocytoma was confirmed by histology. The absence of a phaeochromocytoma in the nonoperated patients was ascertained by a disease-free extended follow-up. We checked the medical charts and/or asked the general practitioner about the patient's health condition with special attention to signs or symptoms suggestive of phaeochromocytoma.

Glucagon stimulation test

After three baseline samples were drawn at 15-minute intervals (-30, -15 and 0 min), 1 mg of glucagon was injected intravenously and its effect on plasma epinephrine and norepinephrine concentrations was measured in blood samples taken one, two and three minutes after injection. Baseline epinephrine and norepinephrine concentrations were calculated as the mean of three baseline samples. Heart rate and blood pressure were recorded every minute with an automated sphygmomanometer until ten minutes after administration of glucagon.

Clonidine suppression test

At least one hour after injection of glucagon, a baseline blood sample was drawn followed 15 minutes later by a second sample and then 0.3 mg clonidine was administered orally. Baseline norepinephrine concentrations were calculated as the mean of the two baseline samples. Blood pressure and heart rate were recorded every 15 minutes with an automated sphygmomanometer until 180 minutes after clonidine administration. A blood sample for determination of norepinephrine concentrations was drawn 180 minutes after intake of clonidine.

Analytical methods

Plasma epinephrine and norepinephrine were assayed by RP-HPLC with fluorimetric detection after solvent extraction and derivatisation with 1,2-diphenylethylenediamine.[8] The inter-assay CV was 6 to 11%. Detection limits were 0.05 nmol/l for plasma epinephrine and norepinephrine.

Statistical methods

Values below the detection limits of the assays were included as having the value of 50% of the detection

limit. The glucagon test was considered positive if plasma norepinephrine was >11.83 nmol/l or if the increase in plasma norepinephrine after glucagon was more than three times the basal values.[5] The clonidine test was considered positive if there was less than 50% reduction in plasma norepinephrine and plasma norepinephrine was >2.95 nmol/l three hours after clonidine administration.[5,9] Data are reported as median (minimum – maximum). Area under curve of the receiver-operator-characteristic (ROC) curves were analysed with SPSS 14.0. P values below 0.05 were considered statistically significant.

RESULTS

Patient characteristics

We included 55 patients of whom 11 (20 %) had a phaeochromocytoma. Patient characteristics are shown in *table 1*. The follow-up period in patients without phaeochromocytoma was 56 (19 to 154) months. Eight patients were on doxazosin during the tests, but none of these patients proved to have a phaeochromocytoma.

Glucagon stimulation test

The glucagon test was not performed in one patient because of hypertension (blood pressure >160/100 mmHg). The results of the glucagon test are shown in *figure 1*. The sensitivity of the glucagon test was 30% and the specificity 100% using cut-off values of 11.83 nmol/l for the norepinephrine peak and a threefold increase (*table 2*). The epinephrine response was highly variable and did not discriminate between patients with and without phaeochromocytoma (*figure 1*). Areas under the ROC curve were 0.691 (95% confidence interval (CI) 0.465 to 0.917; p=0.061) and 0.848 (95% CI 0.696 to 1.000; p=0.001) for the norepinephrine peak and fold-increase, respectively (*figure 2*).

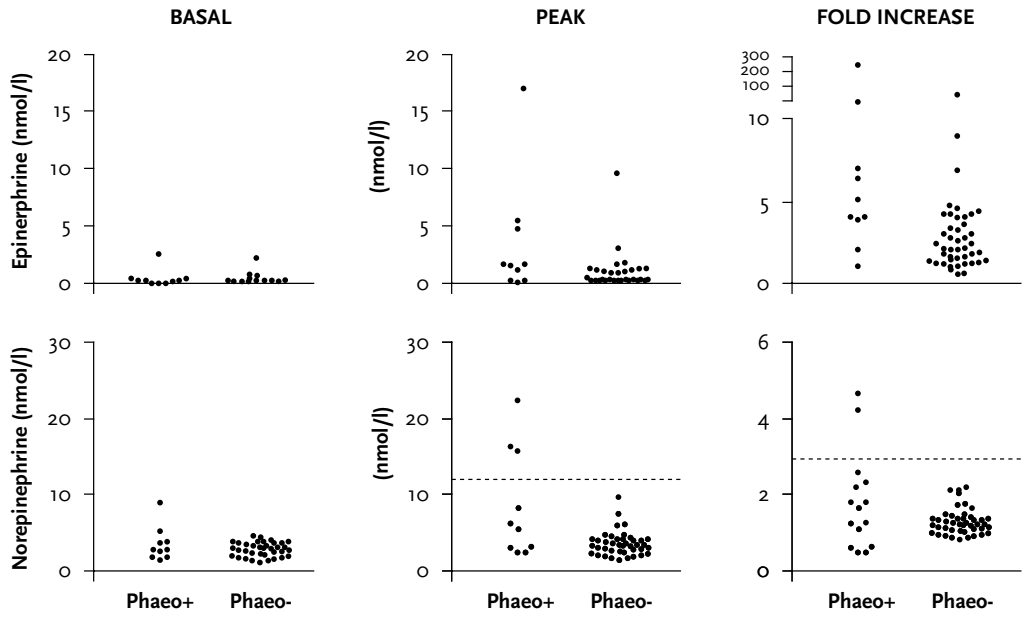
Table 1. *Patient characteristics*

Indication for testing	Male/ female (n)	Age (years)	Phaeochromocytoma n(%)
Clinical suspicion	13/19	47 (19-76)	4 (13)
Genetic predisposition	3/2	32 (19-65)	3 (60)
Adrenal incidentaloma	7/9	57 (43-72)	3 (19)
Recurrence	0/1	69 (69-69)	1 (100)
Total	*23/32*		*11 (20)*

Table 2. *Glucagon stimulation test*

Glucagon test result (n)	Phaeochromocytoma		
	Yes	No	Total
Positive	3	0	3
Negative	7	44	51
Total	*10*	*44*	*54*

Figure 1. *Glucagon stimulation test: Epinephrine and norepinephrine at baseline and after administration of glucagons*



Dotted lines indicate reference values obtained from the literature.[5,6]

Figure 2. *Glucagon stimulation test: Receiver-operator curve for peak (dotted line) and fold-increase (solid line) of norepinephrine*

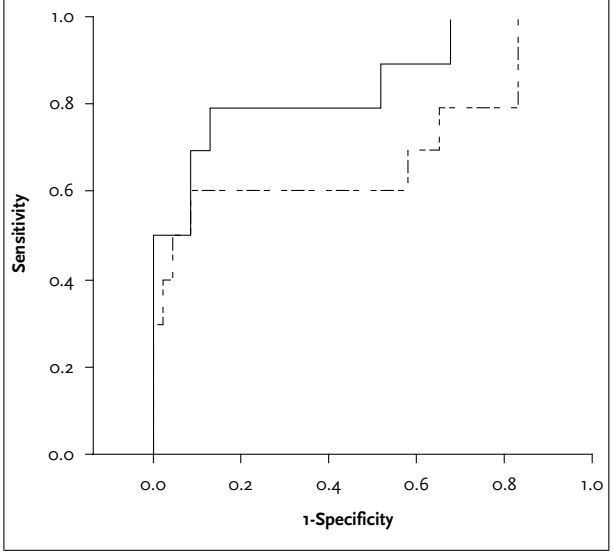
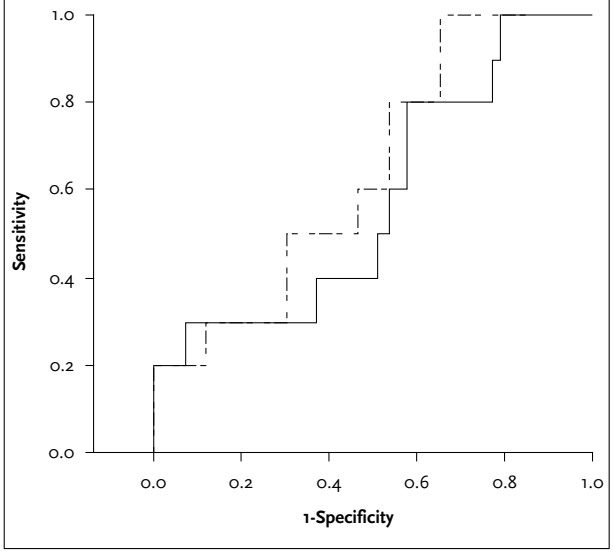


Table 3. *Clonidine suppression test*

Clonidine test result	Phaeochromocytoma		
	Yes	No	Total
Positive	2	3	5
Negative	8	40	48
Total	*10*	*43*	*53*

Figure 4. *Clonidine suppression test: Receiver-operator curve for nadir (dotted line) and relative decrease (solid line) of norepinephrine*



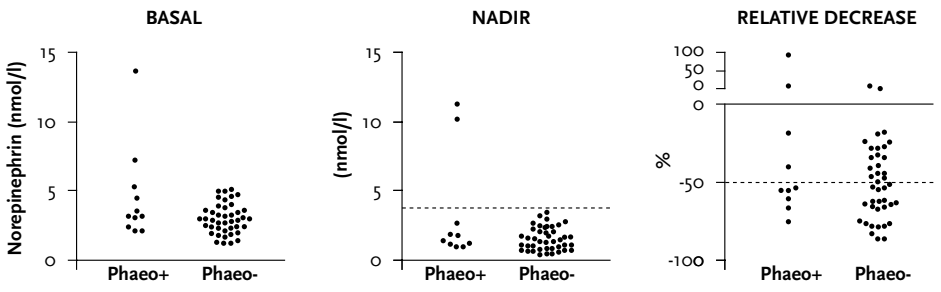
Clonidine suppression test

The clonidine test was not performed in two patients because of hypotension (blood pressure <100/60 mmHg). The results of the clonidine test are shown in *figure 3*. The sensitivity of the clonidine suppression test was 20% and the specificity 93% using the following cut-off values: 50% reduction in plasma norepinephrine after clonidine and plasma norepinephrine >2.95 nmol/l after three hours (*table 3*).[5,9] The areas under the ROC curve were 0.644 (95% CI 0.468 to 0.820; p=0.159) and 0.579 (95% CI 0.380 to 0.778; p=0.440) for the relative norepinephrine decrease and the absolute plasma concentrations after clonidine, respectively (*figure 4*).

DISCUSSION

This is the first study to prospectively evaluate the diagnostic accuracy of two dynamic biochemical tests for the diagnosis of phaeochromocytoma in a group of patients frequently encountered in an outpatient clinic, namely those who had been tested because of suspicious symptoms and signs or predisposing conditions (adrenal incidentaloma or genetic predisposition) and who showed mildly elevated plasma or urine catecholamine levels. The sensitivity and specificity of these tests using reference values from the literature were 30 and 100% for the glucagon test and 20 and 93% for the clonidine test, respectively.

Figure 3. *Clonidine suppression test: Norepinephrine at baseline and after administration of clonidine*



Dotted lines indicate reference values obtained from the literature.[5]

Although the literature reports additional value of the glucagon stimulation test and/or the clonidine suppression test in equivocal cases, our study shows that even in cases with a positive screening test, sensitivity of both tests is low. By lowering the cut-off values for the glucagon test, sensitivity increased from 30 to 90%, but specificity was reduced from 100 to 48%. Given the serious consequences of failure to diagnose a phaeochromocytoma, the glucagon test thus provides no additional value over the measurement of plasma and urine catecholamines and metanephrines in these patients.

The results of the clonidine test in this study differ from some previously published studies on the clonidine test. ROC curve analysis for the clonidine test showed an area under the curve that was not different from 0.5, which indicates that the clonidine test cannot discriminate between patients with and without phaeochromocytoma, whereas others showed a sensitivity of 66 to 100% and a specificity of 93 to 100%.[10,11] Our contrasting findings are probably related to different patient selection criteria. We did not include patients with baseline norepinephrine concentrations above 11.5 nmol/l since this degree of norepinephrine excess is considered pathognomic for phaeochromocytoma. Instead, these patients were not subjected to the clonidine suppression test, but imaging was performed straightaway. Consequently, only four out of ten patients with phaeochromocytoma had baseline norepinephrine concentrations that were above the institutional reference value. In contrast, in the study by Eisenhofer *et al.* 44 out of 48 patients with phaeochromocytoma had increased baseline norepinephrine concentrations and a significant proportion had norepinephrine concentrations above 11.5 nmol/l.[11] Still 16 out of 48 patients with phaeochromocytoma were not detected by a conventional clonidine suppression test. However, with the introduction of plasma normetanephrine measurements during the clonidine test 46 out of 48 patients could be detected.[11] Our observations as well as those of others indicate that the clonidine suppression test without measurement of plasma normetanephrine is not a suitable test for phaeochromocytoma, especially when baseline norepinephrine concentrations are normal or only marginally increased.[5,9,12]

CONCLUSION

The clonidine suppression test without normetanephrine measurements and the glucagon stimulation test are not sensitive enough to safely exclude phaeochromocytoma in patients with mildly elevated plasma or urine catecholamines.

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REFERENCES

1. Stenstrom G, Svardsudd K. Pheochromocytoma in Sweden 1958-1981. An analysis of the National Cancer Registry Data. Acta Med Scand. 1986;220:225-32.
2. Gifford RW, Kvale WF, Maher FT, Roth GM, Priestley JT. Clinical features, diagnosis and treatment of pheochromocytoma: a review of 76 cases. Mayo Clin Proc. 1964;39:281-302.
3. Sheps SG, Maher FT. Histamine and glucagon tests in diagnosis of pheochromocytoma. JAMA. 1968;205:895-9.
4. Lenders JWM, Pacak K, Walther MM et al. Biochemical Diagnosis of Pheochromocytoma: Which Test Is Best? JAMA. 2002;287:1427-34.
5. Grossman E, Goldstein DS, Hoffman A, Keiser HR. Glucagon and clonidine testing in the diagnosis of pheochromocytoma. Hypertension. 1991;17:733-41.
6. Bravo EL, Gifford RW, Jr. Current concepts. Pheochromocytoma: diagnosis, localization and management. N Engl J Med. 1984;311:1298-303.
7. Bravo EL. Evolving concepts in the pathophysiology, diagnosis, and treatment of pheochromocytoma. Endocr Rev. 1994;15:356-68.
8. Van der Hoorn FA, Boomsma F, Man in 't Veld AJ, Schalekamp MA. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection. J Chromatogr. 1989;487:17-28.
9. Elliott WJ, Murphy MB. Reduced specificity of the clonidine suppression test in patients with normal plasma catecholamine levels. Am J Med. 1988;84:419-24.
10. Lenz T, Ross A, Schumm-Draeger P, Schulte KL, Geiger H. Clonidine Suppression Test Revisited. Blood Press. 1998;7:153-9.
11. Eisenhofer G, Goldstein DS, Walther MM, et al. Biochemical Diagnosis of Pheochromocytoma: How to Distinguish True- from False-Positive Test Results. J Clin Endocrinol Metab. 2003;88:2656-66.
12. Sjoberg RJ, Simcic KJ, Kidd GS. The clonidine suppression test for pheochromocytoma. A review of its utility and pitfalls. Arch Intern Med. 1992;152:1193-7.