Universitätsspital Basel

Endokrinologie, Diabetologie und Metabolismus

Pre-test:
Stop desmopressin treatment 24 hours before test start.
Patient should be fasting overnight, but are allowed to drink until 2 hours before test start.
Before test start, participants are settled in a supine position and a catheter is placed in an antecubital vein. Arginine (L-Arginin-Hydrochlorid 21% Braun, B. Braun Melsungen AG) at a dose of 0·5 g/kg body weight (max 40g) diluted in 500 ml NaCl 0·9% is infused over 30 minutes.

Arginine-stimulated copeptin measurements in the differential diagnosis of diabetes insipidus

Dosage calculation for Arginine Infusion

Subject body weight in kg _____x 2.4ml of study drug =_____ml L-Arginine-Hydrochloride 21% diluted with 500 mil NaCL 0.9%

Test date: _____

Infusion start time, 0h: I__I__I:I__I__I
Infusion end time, 0.5h: I__I__I:I__I__I

Comments: _____
_____
_____

Blood Collection

Sample hours	Schedule collection times	Actual collection times	Comments /symptoms*		Visa
0h	I__I__I : I__I__I	I__I__I : I__I__I			
1 h	I__I__I : I__I__I	I__I__I : I__I__I			
Sample hours	**Blood pressure**	**Heart rate**	**Sodium, mmol/L**	**Copeptin, pM/L**	**Visa**
0h	I__I__I__I / I__I__I__I	I__I__I__I			
1 h	I__I__I__I / I__I__I__I	I__I__I__I			

Interpretation of arginine test[1]:
Copeptin <3.8pmol/L: Complete or partial central DI
Copeptin ≥3.8pmol/L: primary polydipsia

Cave:
If patient has severe nausea or is vomiting, copeptin levels have to be interpreted with caution since nausea and vomiting are strong stimuli of copeptin release. Unless copeptin values remain low clearly pointing to DI, we recommend hypertonic saline stimulation test[2] for further differentiation in these situations.

In case of hypernatremia at 1h, copeptin values may be higher due to the additional osmotic stimulus (see hypertonic saline stimulation test).

*Possible symptoms: mild nausea, rarely facial paraesthesia, headache, vertigo, vomitus

1. Winzeler B, Cesana-Nigro N, Refardt J, et al. Arginine-stimulated copeptin measurements in the differential diagnosis of diabetes insipidus: a prospective diagnostic study. *Lancet* 2019; **394**(10198): 587-95.
2. Fenske W, Refardt J, Chifu I, et al. A Copeptin-Based Approach in the Diagnosis of Diabetes Insipidus. *N Engl J Med* 2018; **379**(5): 428-39.

Arginine-stimulated copeptin measurements in the differential diagnosis of diabetes insipidus: a prospective diagnostic study

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Summary

Background Differential diagnosis of diabetes insipidus is challenging. The most reliable approach is hypertonic saline-stimulated copeptin measurements. However, this test is based on the induction of hypernatraemia and requires close monitoring of plasma sodium concentrations. Arginine-stimulated copeptin measurements might provide an alternative, simple, and safe test.

Methods In this prospective diagnostic study, we recruited a development cohort from University Hospital Basel, Basel, Switzerland, and a validation cohort from five centres in Basel, Aarau, Luzern, Bern, and St Gallen, Switzerland, and the University Hospital Würzburg, Würzburg, Germany. For both cohorts, patients were eligible for inclusion if they were aged 18 years or older, were newly referred with polyuria (>50 mL/kg bodyweight per day) or had a known diagnosis of central diabetes insipidus or primary polydipsia. We also recruited a comparator cohort of healthy controls in parallel to each cohort, comprising adults (aged 18 years and older, with normal drinking habits, and no history of polyuria) and children who underwent arginine stimulation to diagnose growth hormone deficiency (children were only included in the comparator cohort to the development cohort as proof of concept). Patients and healthy controls underwent arginine stimulation with measurement of plasma copeptin at baseline and 30, 45, 60, 90, and 120 min. The primary objective in the development cohort was to determine the diagnostic accuracy of plasma copeptin concentrations to discriminate between diabetes insipidus and primary polydipsia, and in the validation cohort was to confirm those results. Adverse effects of the test were monitored in all participants, with tolerability of the test rated using a visual analogue scale (VAS) that ranged from no (0) to maximum (10) discomfort. This trial is registered with ClinicalTrials.gov, number NCT00757276.

Findings Between May 24, 2013, and Jan 11, 2017, 52 patients were enrolled in the development cohort (12 [23%] with complete diabetes insipidus, nine [17%] with partial diabetes insipidus, and 31 [60%] with primary polydipsia) alongside 20 healthy adults and 42 child controls. Between Oct 24, 2017, and June 27, 2018, 46 patients were enrolled in the validation cohort (12 [26%] with complete diabetes insipidus, seven [15%] with partial diabetes insipidus, and 27 [59%] with primary polydipsia) alongside 30 healthy adult controls (two patients in this cohort were excluded from the main analysis because of early vomiting during the test). In the pooled patient and control datasets, median arginine-stimulated copeptin concentrations increased in healthy adult controls (from 5·2 pM [IQR 3·3–10·9] to a maximum of 9·8 pM [6·4–19·6]) and in participants with primary polydipsia (from 3·6 pM [IQR 2·4–5·7] to a maximum of 7·9 pM [5·1–11·8]), but only minimally in those with diabetes insipidus (2·1 pM [IQR 1·9–2·7] to a maximum of 2·5 pM [1·9–3·1]). In the development cohort, a cutoff of 3·5 pM at 60 min provided the highest diagnostic accuracy of 94% (95% CI 84–98). The accuracy of this cutoff in the validation cohort was 86% (95% CI 73–94). By pooling the data from both cohorts, an optimal accuracy of 93% (95% CI 86–97) was reached at a cutoff of 3·8 pM copeptin at 60 min (sensitivity 93%, 95% CI 86–98; specificity 92%, 95% CI 84–100). The test was safe and well tolerated, with median VAS scores of 3·5 (IQR 2–4) in patients with diabetes insipidus, 3 (2–4) in those with primary polydipsia, 1 (1–3) in healthy adults, and 1 (0–5) in healthy children in the pooled participant dataset.

Interpretation Arginine-stimulated copeptin measurements are an innovative test for diabetes insipidus with high diagnostic accuracy, and could be a simplified, novel, and safe diagnostic approach to diabetes insipidus in clinical practice.

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Introduction

Polyuria polydipsia syndrome consists of three main subcategories: central or nephrogenic diabetes insipidus and primary polydipsia.[1] Diabetes insipidus is determined by hypotonic polyuria either due to impaired synthesis (central diabetes insipidus) or action (nephrogenic

Research in context

Evidence before this study

For decades, the diagnostic gold standard for diabetes insipidus was the indirect water deprivation test. This test, which dates back to 1970 and was based on data from only 36 patients, was never prospectively validated; therefore, unsurprisingly, it has little diagnostic accuracy. A previous attempt to improve its diagnostic accuracy by incorporation of direct measurement of the antidiuretic hormone vasopressin did not hold promise, mainly due to technical limitations of the assay. Our group has recently shown that copeptin—the stable surrogate marker of vasopressin—measured after hypertonic saline stimulation is a reliable test in the differential diagnosis of polyuric states. However, this test needs sodium overstimulation and, therefore, close monitoring. Several caveats must be considered and an easier way to stimulate copeptin without inducing high sodium levels would be ideal. Arginine infusion is known to stimulate anterior pituitary hormones such as growth hormone and so is widely used as a simple and well tolerated tool to diagnose growth hormone deficiency. We hypothesised that arginine infusion could be a useful and simple test to stimulate copeptin and diagnose diabetes insipidus.

Added value of this study

This study shows that arginine is a potent stimulus of the posterior pituitary gland and that arginine-stimulated copeptin measurements are an accurate test for diabetes insipidus. We found that a copeptin cutoff of 3·8 pM/L at 60 min after arginine infusion had an accuracy of 93% to diagnose diabetes insipidus in a dataset of patients with diabetes insipidus and primary polydipsia. The test was safe and had an acceptable tolerability profile, with mild nausea being common, but adverse effects such as vertigo, headache, and malaise (previously described in up to 70% of patients during hypertonic saline stimulation) were negligible during arginine stimulation.

Implications of all the available evidence

Arginine-stimulated copeptin measurements show a similar diagnostic accuracy to the hypertonic saline approach but is more practical in clinical routine and associated with fewer adverse effects and risks. We expect that the convenient arginine stimulation test will become the standard diagnostic test for diabetes insipidus.

diabetes insipidus) of the antidiuretic hormone vasopressin.[2] Primary polydipsia is characterised by excessive fluid intake, which seems to be a growing occurrence in individuals who are lifestyle conscious.[3] Discriminating between these subcategories is crucial because treatment for each condition differs considerably.[4]

For many decades, the indirect water deprivation test has remained the accepted diagnostic gold standard for differentiating between polyuric states, even though its diagnostic criteria were based on data from only 36 patients[5,6] and yielded a poor overall diagnostic accuracy of 70%.[7] Additional measurement of plasma vasopressin concentrations to enhance the sensitivity of the water deprivation test[8] did not overcome the limitations of the test, mainly because of technical restrictions of the vasopressin assay.[9] Copeptin, the C-terminal segment of the vasopressin precursor peptide, is a novel and stable surrogate marker of vasopressin and is easily measured with a sandwich immunoassay.[10,11] We have published the use of hypertonic saline-stimulated copeptin measurements in the diagnosis of polyuria polydipsia syndrome[12] as a replacement for the water deprivation test for discrimination between diabetes insipidus and primary polydipsia. However, the hypertonic saline infusion test is based on the induction of hypernatraemia and therefore has several caveats including the adverse effects associated with an increase in sodium. The test requires close monitoring of sodium concentrations, and is contraindicated in some patients (eg, those with a history of heart failure or epilepsy).

Arginine is known to stimulate the anterior pituitary gland to secrete various hormones—such as prolactin[13,14] and growth hormone.[15] Therefore, arginine stimulation is widely used as a simple and well tolerated tool to diagnose growth hormone deficiency,[16,17] especially in children.[18]

In accordance with the effects of other growth hormone secretagogues (eg, hexarelin),[19,20] we hypothesised that arginine could also stimulate the posterior pituitary (ie, to release vasopressin) and might therefore provide a simple, alternative diagnostic test in the differential diagnosis of diabetes insipidus.

Methods

Study design and participants

This prospective diagnostic study was undertaken at the University Hospital Basel, Basel, Switzerland, and two separate cohorts were recruited: the development cohort, which was to be a proof of concept cohort to test the study hypothesis; and the validation cohort, to confirm the findings of the development cohort. First, we recruited the development cohort and a comparator control cohort from University Hospital Basel. Newly referred patients with polyuria (>50 mL/kg bodyweight per day) or patients under ongoing clinical care with a known diagnosis of central diabetes insipidus or primary polydipsia were eligible if they were aged 18 or older. Before study enrolment, an indirect water deprivation test and a single random copeptin measurement were done. The copeptin measurement was done to identify patients with nephrogenic diabetes insipidus,[21] who were excluded from the study. A cohort of healthy controls was recruited in parallel, comprising healthy adults and children under investigation for short stature (with no specific age limit).

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Healthy adults were eligible if they were aged 18 years or older, had normal drinking habits, and no history of polyuria. For such children, arginine stimulation was routinely done to exclude growth hormone deficiency according to international guidelines.[18] Exclusion criteria for children were evidence of altered drinking habits and diuresis and acute illness. Detailed eligibility criteria are in the appendix (p 2). Second, we recruited the validation cohort and a second control cohort of healthy adults from five centres in Basel, Aarau, Luzern, Bern, and St Gallen, Switzerland and from the University Hospital Würzburg, Würzburg, Germany, with the same inclusion and exclusion criteria. All tests were done at University Hospital Basel.

The local ethics committee approved the study protocol. Written informed consent was obtained from all study participants or the parents of children.

Procedures

All participants underwent a standardised arginine infusion starting at 0800 h, after an overnight fast of 8 h and fluid restriction for 2 h (children were allowed to drink water until test start). Patients on desmopressin treatment discontinued their medication at least 24 h before the test. 30 min before test start, participants were settled in a supine position and a catheter was placed in an antecubital vein. Arginine (L-arginine-hydrochloride 21%, Braun, B Braun Melsungen AG, Melsungen, Germany), at a dose of 0·5 g/kg bodyweight, diluted in 500 mL of 0·9% sodium chloride solution, was infused over 30 min. At baseline and 30, 45, 60, 90, and 120 min after the start of arginine infusion, blood pressure and pulse rate were monitored and blood was drawn for copeptin measurement.

Plasma copeptin concentrations were measured in one batch with a commercial automated immunofluorescence assay (B.R.A.H.M.S Copeptin-proAVP KRYPTOR, Brahms, Thermo Scientific Biomarkers, Hennigsdorf, Germany; appendix p 2).[10]

At baseline and at the end of the test (at 120 min) routine laboratory measurements were done of plasma and urine samples with automated biochemical analyses in the University Central Laboratories (University Hospital Basel, Basel, Switzerland). In the validation cohort, glucose was additionally measured at each timepoint.

During the test, adverse effects were strictly documented. Participants scored their symptoms and rated the overall test burden using a visual analogue scale (VAS) that ranged from 0 to 10, where 0 is no discomfort and 10 is maximum discomfort (appendix p 9).

A final diagnosis of diabetes insipidus or primary polydipsia was done over two steps after study termination for the patients in each cohort. Before study inclusion, we adjudicated all patients to a working diagnosis of partial or complete central diabetes insipidus or primary polydipsia. In the absence of a clearly defined diagnostic gold standard, diagnosis was made after careful, comprehensive assessment according to three diagnostic components: results of the indirect water deprivation test according to the protocol of Miller and colleagues,[5] patients' history, and response to treatment with desmopression or water restriction (applied diagnostic criteria are in the appendix [p 3]).

After termination of the study, the initial diagnoses were reviewed and confirmed by two experts in the field applying the same diagnostic principles (and masked to copeptin concentrations; BW and MC-C). In all cases of uncertainty or discordance, participant-level data were sent to a third external independent expert and results were discussed until agreement was reached.

See Online for appendix

Outcomes

The primary objective in the development cohort was to assess the diagnostic performance of copeptin concentrations at 30, 45, 60, 90, and 120 min after arginine stimulation to discriminate between patients with central diabetes insipidus and those with primary polydipsia. The primary endpoint was the diagnostic accuracy of copeptin concentration at each measurement after arginine stimulation.

The primary objective in the validation cohort was to validate the diagnostic performance of the best cutoffs of copeptin after arginine stimulation derived from the development cohort.

The primary objective in the pooled patient dataset (ie, the development cohort plus the validation cohort) was to re-assess the diagnostic performance of copeptin concentrations at each timepoint to discriminate between patients with central diabetes insipidus and those with primary polydipsia.

Further objectives in the pooled patient dataset and pooled healthy control dataset (ie, all participating healthy adult controls) were the description of the course of copeptin after arginine stimulation, comparison of vital signs and electrolyte concentrations before and after arginine stimulation, and assessment of tolerability of the test.

Statistical analysis

We estimated the sample size of the development cohort to be able to show a diagnostic accuracy of plasma copeptin concentrations to discriminate between patients with primary polydipsia and those with central diabetes insipidus of at least 80% (lower level of two-sided 95% CI). A total of 40 evaluable patients with either primary polydipsia or diabetes insipidus was needed to achieve a power of at least 80%. A similar sample size was considered appropriate for the validation cohort.

We assessed the time course of copeptin concentration after arginine stimulation exploratively by means of boxplots and trellis plots in the pooled datasets of patients and healthy adults and children and for subgroups of patients with complete or partial diabetes insipidus and primary polydipsia.

We analysed data for patients with diabetes insipidus or primary polydipsia for whom at least one copeptin measurement after arginine stimulation was available. Copeptin concentrations of lower than 0·1 pM could not be determined with precision, and so these values were set to 0·1 pM.

We assessed diagnostic accuracy for discrimination of patients with diabetes insipidus or primary polydipsia for each measurement time on the basis of the best copeptin cutoff for that respective timepoint derived from the patient datasets that resulted in the best combination of specificity and sensitivity.[22] We used receiver operating characteristic (ROC) area under the curve (AUC) to calculate these results. Additionally, we tested the diagnostic performance of copeptin using the maximum concentration recorded between 30 min and 120 min after arginine stimulation for each patient.

We did a prespecified subgroup analysis of diagnostic accuracy excluding patients with complete diabetes insipidus—who are typically easier to diagnose—using the same procedures.

For patients and healthy adults, we summarised vital signs and electrolyte concentrations using median and IQRs. We used linear mixed-effects regression models to test for a difference between diagnoses, measurement time, and their interaction.

We calculated frequencies and proportions of participants with adverse effects for each group, including all participants who indicated any sign of adverse effects (at least one value above 0, as indicated by VAS). We assessed overall subjective level of discomfort during the test for each group in the pooled dataset.

We did post-hoc analyses for the association between clinical variables (baseline sodium and osmolality, age, sex, body-mass index [BMI], and nausea during the test) and copeptin response. Furthermore, we did a post-hoc head-to-head comparison in 60 patients who participated in the study and in a parallel hypertonic saline-stimulation study.[12] Statsistical details of this head-to-head comparison are in the appendix (p 4).

We did all analyses using the statistical software package R (R Foundation for Statistical Computing). Full details of statistical analyses are in the appendix (p 4). The study was registered on ClinicalTrials.gov, identifier NCT00757276.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The first and the last author had access to all the data and had final responsibility for the decision to submit for publication.

Results

Between May 24, 2013, and Jan 11, 2017, 52 patients from Basel, 31 (60%) with primary polydipsia and 12 (23%) with complete and nine (17%) with partial diabetes insipidus, and 20 healthy adult controls and 42 child controls with short stature were enrolled in the development cohort. And between Oct 24, 2017, and June 27, 2018, 46 adult patients, 27 (59%) with primary polydipsia and 12 (26%) with complete and seven (15%) with partial diabetes insipidus, and 30 healthy adults were recruited for the validation cohort (results of the water deprivation test for diagnoses are in the appendix [p 5]) Two patients in the validation cohort were excluded from the main analysis because of early vomiting during the test, but were included in the tolerablity analysis.

Baseline demographic and clinical characteristics of patients in both cohorts are in table 1. Patients with primary polydipsia tended to be younger than those with diabetes insipidus, especially in the validation cohort, and were predominantly female (table 1). For patients with complete diabetes insipidus, for seven (58%) of 12 in the development cohort and nine (82%) of 11 in the validation cohort, their diabetes insipidus was primarily due to a pituitary lesion or history of pituitary surgery, and for four (33%) in the development cohort and two (18%) in the validation cohort, their diabetes insipidus was congenital. Partial diabetes insipidus was linked to a pituitary lesion in five (56%) of nine patients in the development cohort and four (67%) of six in the validation cohort, and was otherwise idiopathic. The pooled control dataset included 50 healthy adults, of whom 23 (46%) were male, with a mean age of 30·0 years (SD 9·9) and mean BMI of 23·9 kg/m² (SD 4·4); and 42 children, of whom 24 (57%) were male, with a mean age of 8·5 years (SD 2·9). Growth hormone deficiency was present in ten (24%) children. Other comorbidities for children are in the appendix (p 4).

In the pooled patient dataset, the median copeptin values for those with primary polydipsia doubled after arginine stimulation, from 3·6 pM (IQR 2·4–5·7) at baseline to a maximum of 7·9 pM (5·1–11·8) at 120 min (p<0·0001). By contrast, copeptin concentrations from patients with diabetes insipidus increased only slightly after arginine stimulation (median 2·1 pM [IQR 1·9–2·7] at baseline) to a maximum of 2·5 pM (1·9–3·1) at 60 min (p=0·0013; figure 1). In the subgroups of patients with complete and partial diabetes insipidus, we observed only a small change in copeptin concentrations for those with complete diabetes insipidus (from 1·9 pM [IQR 1·6–2·4] to a maximum at 60 min of 2·2 pM [1·6–2·8]; p=0·0032), whereas for those with partial diabetes insipidus, copeptin concentrations increased from 2·5 pM (IQR 2·2–3·0) to a maximum at 120 min of 3·3 pM (2·9–3·9; p=0·0035; figure 2).

In healthy adults in the pooled control dataset, the median baseline copeptin concentration was 5·2 pM (IQR 3·3–10·9), which increased in the first 60 min after arginine stimulation to 9·8 pM (IQR 6·4–19·6; p<0·0001; figure 3A). In children in the control dataset, median copeptin concentrations increased from baseline at 4·3 pM (IQR 3·2–6·0) to 6·5 pM (IQR 4·7–8·5;

	Development cohort			Validation cohort		
	Complete diabetes insipidus (n=12)	Partial diabetes insipidus (n=9)	Primary polydipsia (n=31)	Complete diabetes insipidus (n=11)	Partial diabetes insipidus (n=6)	Primary polydipsia (n=27)
Demographic						
Age, years	41 (14·9)	37·8 (9·6)	35·1 (13·4)	53·5 (14·5)	45·7(15·1)	35·5 (10·5)
Sex						
Male	5 (42%)	4 (44%)	9 (29%)	5 (46%)	2 (33%)	7 (26%)
Female	7 (58%)	5 (56%)	22 (71%)	6 (54%)	4 (67%)	20 (74%)
Body-mass index, kg/m²	26 (5·6)	24·9 (3·1)	23·9 (5·1)	29·2 (6·9)	26·2 (3·6)	26·2 (6·5)
Clinical						
Pituitary lesion	7 (58%)	5 (56%)	1 (3%)	9 (82%)	4 (67%)	2 (7%)
Adenoma	3 (25%)	1 (11%)	1 (3%)	2 (18%)	2 (33%)	1 (4%)
Craniopharyngioma	2 (16%)	0	0	3 (27%)	0	0
Rathke cleft cyst	0	3 (33%)	0	0	0	0
Germinoma	1 (8%)	0	0	1 (9%)	0	0
Meningiomas	0	1 (11%)	0	0	0	0
Langerhans cell histiocytosis	1 (8%)	0	0	1 (9%)	0	0
Sarcoidosis	0	0	0	1 (9%)	1 (17%)	0
Hypophysitis	0	0	0	1 (9%)	1 (17%)	1 (4%)
History of pituitary surgery	6 (50%)	5 (56%)	1 (3%)	5 (46%)	2 (33%)	0
Familial diabetes insipidus	4 (33%)	0	0	2 (18%)	0	0
Idiopathic diabetes insipidus	1 (8%)	5 (56%)	0	0	2 (33%)	0
Anterior pituitary deficiency	6 (50%)	5 (56%)	1 (3%)	7 (64%)	3 (50%)	1 (4%)
Depression	1 (8%)	0	10 (32%)	0	0	3 (11%)
Schizophrenia	1 (8%)	0	1 (3%)	0	0	0
Renal insufficiency	0	0	1 (3%)	0	0	0
Cardiovascular disease	1 (8%)	1 (10%)	1 (3%)	1 (9%)	0	2 (7%)
Medication and noxae at baseline						
Desmopressin	8 (67%)	5 (56%)	1 (3%)	11 (100%)	5 (83%)	2 (7%)
Hydrocortisone	5 (41%)	1 (11%)	1 (3%)	6 (55%)	2 (33%)	0
Levothyroxine	5 (41%)	3 (33%)	1 (3%)	7 (64%)	2 (33%)	2 (7%)
Testosterone	4 (33%)	4 (44%)	2 (7%)	4 (36%)	2 (33%)	5 (19%)
Hormonal contraceptives	3 (25%)	0	5 (16%)	0	1 (17%)	4 (15%)
Growth hormone	1 (8%)	1 (11%)	0	2 (18%)	0	0
Antidepressant	1 (8%)	1 (11%)	3 (10%)	0	1 (17%)	2 (7%)
Other psychotropic drug	2 (16%)	0	2 (7%)	0	0	1 (4%)
Other medication	6 (50%)	2 (22%)	2 (7%)	4 (36%)	1 (17%)	9 (33%)
Smoking	5 (42%)	2 (22%)	7 (23%)	1 (9%)	2 (33%)	6 (22%)
Alcohol (>2 glasses per day)	1 (8%)	0	1 (3%)	0	0	0

Data are mean (SD) or n (%).

Table 1: **Baseline characteristics of patient dataset**

p<0·0001) by 60 min after arginine stimulation (figure 3B). Notably, no association was seen between growth hormone deficiency and maximum copeptin concentrations achieved by children (data not shown).

In the development cohort, diagnostic performance of the best copeptin cutoffs for each measurement are summarised in the appendix (p 6). The highest diagnostic accuracy was observed for copeptin measured 60 min after arginine stimulation (94%, 95% CI 84–98), using a cutoff of 3·5 pM (sensitivity 91%, specificity 97%). The AUC for the corresponding ROC was 0·94 (95% CI 0·87–1·00; figure 4A). By using the maximum copeptin concentration measured between 30 min and 120 min for each patient in the development cohort, the diagnostic performance (cutoff 3·5 pM, accuracy 88% [95% CI 84–92], sensitivity 85%, specificity 90%) was inferior to that resulting from just copeptin concentrations measured at 60 min.

In a subgroup analysis of the development cohort excluding patients with complete diabetes insipidus, the highest diagnostic accuracy was observed for copeptin concentrations measured at 60 min after arginine stimulation (93%, 95% CI 81–98) using a cutoff of 3·4 pM (sensitivity 78%, specificity 97%; appendix p 6).

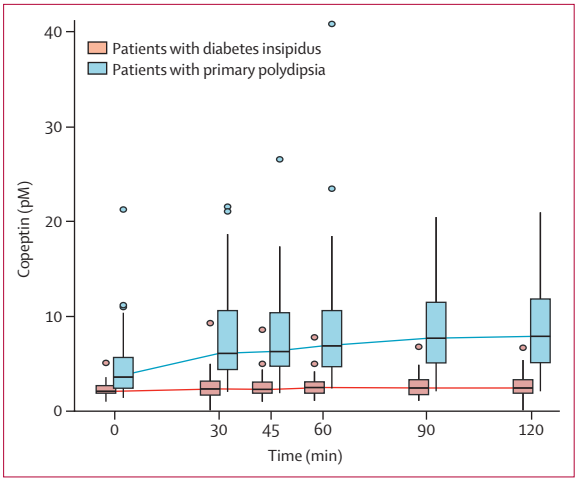

Figure 1: **Copeptin concentrations after arginine stimulation in patients with diabetes insipidus (complete and partial) and primary polydipsia, in the pooled patient dataset**

Boxes span the IQR, the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1·5 times the IQR. All other values are considered to be outliers and plotted as individual points. For better presentation, the y axis is shown up to 40 pM; hence, nine outliers with concentrations above 40 pM are not shown. Pooled patient dataset includes all patients in both the development and validation cohorts.

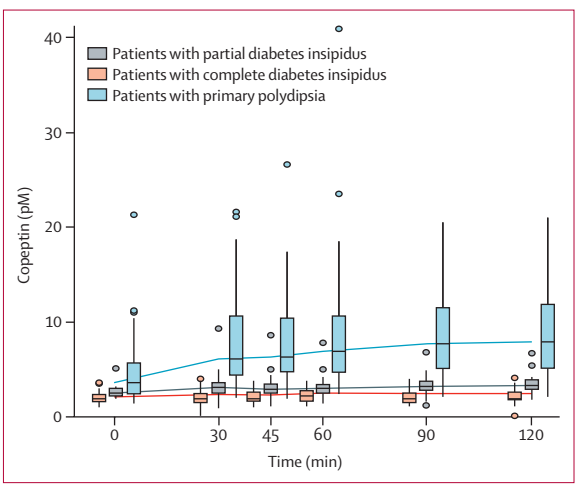

Figure 2: **Copeptin concentrations after arginine stimulation in patients with partial diabetes insipidus, complete diabetes insipidus, and primary polydipsia in the pooled patient dataset**

Boxes span the IQR, the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1·5 times the IQR. All other values are considered to be outliers and plotted as individual points. For better presentation, the y axis is shown up to 40 pM; hence, nine outliers with concentrations above 40 pM are not shown. Pooled patient dataset includes all patients in both the development and validation cohorts.

In the validation cohort, diagnostic performance of the best cutoff derived from the development cohort (copeptin 3·5 pM at 60 min) resulted in diagnostic accuracy of 86% (95% CI 73–94), with a sensitivity of 77% and specificity of 93%, which was slightly lower than in the development cohort. The AUC for the corresponding ROC was high (0·96, 95% CI 0·90–1·00; figure 4B). Diagnostic accuracy of the best cutoff to differentiate between partial diabetes insipidus and primary polydipsia derived from the development cohort (copeptin 3·4 pM at 60 min) was 88% (95% CI 0·72–0·96), with a sensitivity of 93% and specificity of 67%; again, the corresponding ROC AUC value was high (0·95, 95% CI 0·88–1·00).

For the pooled patient dataset, the highest diagnostic accuracy for differentiating between diabetes insipidus and primary polydipsia was observed for a copeptin cutoff of 3·8 pM, measured at 60 min after arginine stimulation: 93% (95% CI 86–97), with a sensitivity of 93% and specificity of 92%. The AUC for the corresponding ROC was 0·95 (95% CI 0·92–0·99; figure 4C, table 2). Likewise, the highest diagnostic accuracy for differentiating between partial diabetes insipidus and primary polydipsia was found for a copeptin cutoff of 3·8 pM, measured at 60 min after arginine stimulation (90% [95% CI 82–96]; sensitivity 93%, specificity 80%). The AUC for the corresponding ROC was 0·91 (95% CI 0·83–0·99; appendix p 6).

Overall, clinical parameters of patients and healthy adults when assessed in the pooled patient and control datasets were in the normal range and remained stable during the test (table 3). Patients with diabetes insipidus had lower median urine osmolarities than patients with primary polydipsia, and their median plasma sodium concentration and plasma osmolality increased slightly during the test, whereas these values remained stable in patients with primary polydipsia (table 3). Seven patients (all with diabetes insipidus) had mild hypernatraemia (range 146–149 mmol/L) at the end of the test.

Overall, arginine stimulation was well tolerated in all three study populations, except for the two patients in the validation cohort who were excluded from the main analysis due to early vomiting. In both pooled cohorts, participants indicated a low level of discomfort during arginine stimulation, with median VAS scores of 3·5 (IQR 2–4) in patients with diabetes insipidus, 3 (2–4) in those with primary polydipsia, 1 (1–3) in healthy adults, and 1 (0–5) in healthy children. Besides thirst, the most common adverse effect during the test was mild nausea, reported by 58% (23 of 40) of patients with diabetes insipidus, 41% (24 of 58) of those with primary polydipsia, 26% (13 of 50) of healthy adults, and 24% (ten of 42) of healthy children. Overall for all pooled participants, median VAS scores for nausea were 1 (range 0–10) for patients with diabetes insipidus, 1 (0–9) in those with primary polydipsia, 0 (0–4) for healthy adults, and 0 (0–7) in healthy children. Other adverse effects were vertigo (five patients [n=1 diabetes insipidus, n=4 primary polydipsia] and three healthy adults), facial or oral paraesthesia (five patients [n=4 diabetes insipidus, n=1 primary polydipsia] and two healthy adults), and transient headache without requiring pain medication (four patients [n=2 diabetes insipidus, n=2 primary polydipsia] and one healthy adult). Nine of 190 participants

(seven patients [n=4 diabetes insipidus, n=3 primary polydipsia], one healthy adult, and one child) vomited during the test, including the two who vomited before the first measurement. Two patients (n=2 diabetes insipidus) had symptomatic hypotonus, of which one case was judged to be due to lack of compliance with hydrocortisone treatment. Finally, one patient with diabetes insipidus had unclear transient malaise leading to premature termination of the test after 60 min.

In our post-hoc analyses, we found no evidence for an association between plasma sodium concentration or osmolality at test start, age, sex, BMI, and nausea with the copeptin response (appendix p 7). In the post-hoc head-to-head comparison between arginine-stimulated and hypertonic saline-stimulated copeptin measurement, the diagnostic accuracy of the arginine stimulation test was 93% (95% CI 84–98), compared with 100% (95% CI 94–100) with the hypertonic saline infusion test (appendix p 8). Nausea was common during both tests, but other adverse effects of headache, vertigo, and malaise occurred in over 70% of patients during hypertonic saline infusion and were negligible during arginine stimulation (≤5%; appendix p 9).

Discussion

In this study we found that arginine infusion is a non-osmotic stimulus of the posterior pituitary, as shown by increased copeptin concentrations in healthy adults and children, and that arginine-stimulated copeptin concentrations can differentiate between patients with central diabetes insipidus and primary polydipsia with high diagnostic accuracy.

The amino acid arginine is an endogenous precursor of nitric oxide, which is an important signalling molecule in many endocrine regulatory pathways.[23] Administration of oral or intravenous arginine has been shown to promote various metabolic effects; insulin is released, plasma concentrations of free fatty acids decrease, and release of hormones of the pituitary gland (such as, growth hormone, prolactin, and thyroid stimulating hormone), are stimulated.[14,15,24] Therefore, arginine infusion is one of the recommended standard growth hormone provocation tests for diagnosing growth hormone deficiency in children with small stature.[18,25] Other growth hormone secretagogues (eg, hexarelin) have been shown to stimulate release of hypothalamic vasopressin in vitro.[19,20] Our results indicate that arginine is not only a potent stimulus of the anterior, but also of the posterior pituitary gland. Arginine might exert its action on vasopressin via the L-arginine–nitric oxide pathway.[26]

In this study, arginine-stimulated copeptin concentrations reliably discriminated between patients with diabetes insipidus and those with primary polydipsia with an AUC of 0·95 by use of a cutoff of 3·8 pM at 60 min. The discrimination between primary polydipsia and partial diabetes insipidus is a challenging and clinically relevant differential diagnosis;[4] the diagnostic accuracy of arginine-stimulated copeptin measurements was also high in this setting.

The value of direct copeptin measurement in the whole spectrum of polyuria polydipsia syndrome has previously been compared with osmotic stimulation—ie, the water

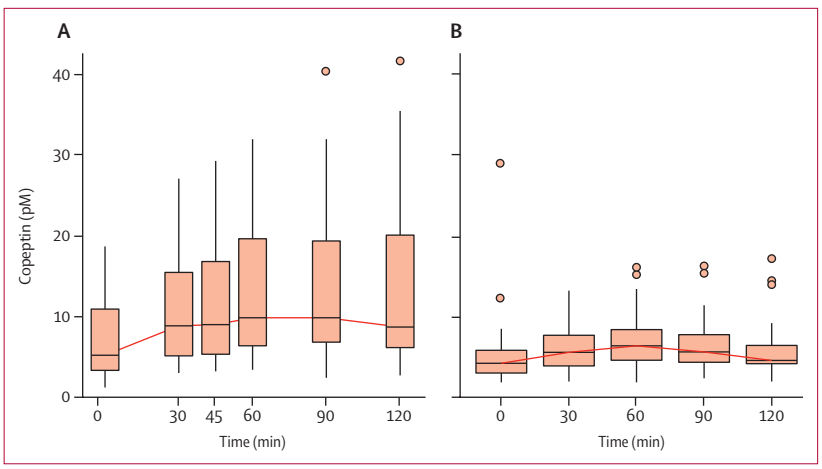

Figure 3: **Copeptin concentrations after arginine stimulation in (A) the pooled dataset of healthy adults and (B) children**
Boxes span the IQR, the thick horizontal line is the median. Whiskers are the most extreme values lying within the box edge and 1·5 times the IQR. All other values are considered to be outliers and plotted as individual points. For better presentation, the y axis is shown up to 40 pM; hence, 16 outliers with concentrations above 40 pM are not shown.

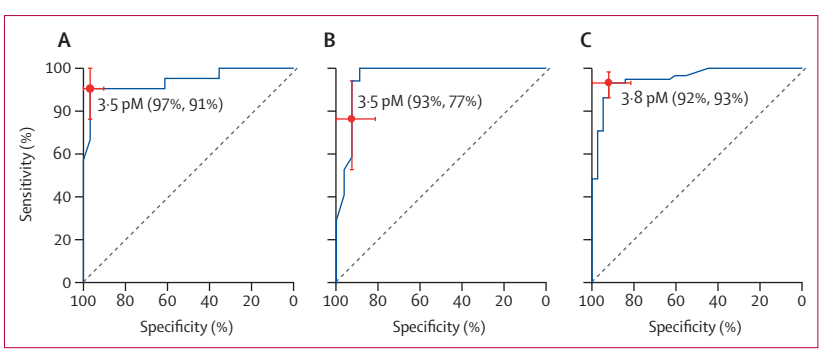

Figure 4: **Receiver-operating-characteristic area under the curve and best copeptin cutoff at 60 min after arginine stimulation in the (A) development cohort (B) validation cohort and (C) pooled patient dataset**
In panel B, the best cutoff derived from the development cohort is being validated in the validation cohort. Data in parentheses are specificity and sensitivity.

	Area under the curve	Best cutoff	Sensitivity	Specificity	Diagnostic accuracy
30 min	0·93 (0·87–0·98)	4·1 pM	80% (70–91)	95% (87–100)	87% (78–92)
45 min	0·94 (0·89–0·99)	3·8 pM	89% (80–96)	92% (82–100)	91% (83–95)
60 min	0·95 (0·91–0·99)	3·8 pM	93% (86–98)	92% (84–100)	93% (86–97)
90 min	0·95 (0·91–0·99)	4·2 pM	86% (77–95)	94% (86–100)	90% (82–95)
120 min	0·95 (0·90–0·99)	4·2 pM	86% (77–95)	92% (81–100)	86% (81–94)

95% CIs are in parentheses.

Table 2: **Best copeptin cutoffs at different timepoints after arginine stimulation for highest diagnostic accuracy for differentiating between diabetes insipidus and primary polydipsia in the pooled patient population**

	Diabetes insipidus (n=38)		Primary polydipsia (n=58)		p value*, 0 min	p value†, time interaction 120 min	Control cohort, adults (n=50)	
	0 min	120 min	0 min	120 min			0 min	120 min
Blood pressure systolic, mm Hg	123 (113–135)	123 (113–132)	117 (103–126)	115 (104–122)	0·002	0·56	120 (113–127)	116 (108–125)
Blood pressure diastolic, mm Hg	78 (72–84)	74 (70–84)	73 (67–81)	71 (65–76)	0·011	0·48	72 (68–80)	68 (64–76)
Heart rate, beats per min	67 (61–79)	70 (61–78)	64 (58–71)	65 (55–70)	0·032	0·39	73 (58–79)	66 (59–73)
Plasma sodium concentration, mmol/L	142 (140–144)	143 (141–146)	140 (139–142)	140 (139–141)	0·002	0·008	141 (139–141)	140 (138–141)
Plasma osmolality, mmol/kg	292 (289–296)	297 (293–304)	291 (287–293)	291 (289–295)	0·011	0·020	291 (287–294)	291 (288–293)
Plasma glucose concentration, mmol/L‡	5·2 (4·5–5·3)	5 (4·5–5·3)	5 (4·9–5·7)	4·8 (4·7–5·2)	0·90	0·30	4·9 (4·8–5·5)	4·8 (4·5–5·1)
Urine osmolality, mmol/kg	195 (118–439)	286 (167–381)	560 (297–742)	531 (450–577)	<0·0001	0·70	789 (630–900)	567 (535–594)

Data are median (IQR). p values are derived from multiple linear mixed-effects regression models. *Comparison at 0 min of patients with primary polydipsia to those with diabetes insipidus. †Interaction: time (120 to 0 min) × diagnosis (primary polydipsia *vs* diabetes insipidus). ‡Values only available in validation cohort.

Table 3: **Clinical and laboratory parameters before and after arginine stimulation in the pooled patient population and adults from the pooled control population**

deprivation test and hypertonic saline infusion.[7,12,21] Water deprivation alone is often not effective.[7] More accurate differentiation (AUC 0·97) between primary polydipsia and diabetes insipidus can be gained with hypertonic saline infusion.[12] Unfortunately, induction of hypernatraemia is associated with adverse effects and can be critical in patients with a history of heart failure or epilepsy.[12,27] Moreover, this test needs close monitoring of plasma sodium concentrations, which makes it cumbersome in clinical routine. The arginine test reported here has similar diagnostic accuracy but is clinically simpler and safer.

During arginine stimulation, clinical and laboratory parameters remained generally stable and in the normal range. Seven patients (all with diabetes insipidus) had mild hypernatraemia (range 146–149 mmol/L) at the end of the test. Overall, the test burden was low, although nausea was a frequent symptom during arginine stimulation. Nausea was typically mild and of transient nature, as were other adverse effects, which were observed in single cases (eg, vertigo, headache, or malaise). Compared with hypertonic saline stimulation, which is associated with adverse effects in many patients,[12] we found the tolerability profile of arginine stimulation to be more attractive among participants. The finding is best shown by the post-hoc head-to-head comparison in which the frequency of nausea was similar in both tests, but during hypertonic saline stimulation over 70% of patients had vertigo, headache, or malaise, or a combination of these compared with 5% or fewer during arginine stimulation.

Further advantages of arginine-stimulated copeptin measurements over the water deprivation and hypertonic saline infusion procedures are the improved feasibility and safety at a similar diagnostic accuracy as hypertonic saline stimulation. According to our data, one measurement of copeptin at 60 min after arginine stimulation is sufficient for the differential diagnosis of diabetes insipidus, making this test protocol even more simple than the other procedures.

Comparing patients with complete versus partial diabetes insipidus, those with complete disease showed a negligible copeptin increase, whereas those with partial disease tended to have greater increased copeptin concentrations (both at baseline and after stimulation). However, because treatment of complete versus partial diabetes insipidus does not differ, this distinction is not clinically relevant.

We did not find any evidence that clinical variables such as age, sex, or BMI affect copeptin response. Similarly, basal sodium or nausea were not associated with an altered copeptin response. These findings are important because both hypernatraemia and nausea are well known stimuli.[28] Notably, our patients were normonatraemic at test start and, if nausea occurred, it was generally mild and transient; hence, we were not likely to see such associations in our population. For future test application, we suggest our results should be interpreted with caution in case of hypernatraemia, severe nausea, or vomiting. Unless copeptin concentrations remain low in keeping with a diagnosis of diabetes insipidus, we recommend hypertonic saline stimulation for further differentiation in patients with nausea and vomiting.

Our study had several limitations. First, although diagnosis of diabetes insipidus and primary polydipsia has been made according to a previously assessed[5] and widely used approach in clinical routine, patients might have been misclassified. Second, in terms of arginine dosing, we adopted the same test protocol as for growth hormone stimulation[14] without a previous dose-response study. Third, because the study was initially designed as a proof-of-concept study, the number of patients in the development cohort was quite small. The best cutoff derived from this cohort was found to be suboptimal when tested in validation cohort. Nevertheless, the ROC AUC was still very high, strengthening our assumption that this test has a high potential to differentiate between diabetes insipidus and primary polydipsia. Therefore, we recommend a cutoff based on pooled data from both cohorts. Finally, the head-to-head comparison between

the arginine stimulation test and the hypertonic saline infusion test was retrospective and not prespecified, and so further testing of the performance of both tests in a prospective head-to-head evaluation should be done.

The strengths of our study include the novelty of this diagnostic approach to diabetes insipidus, the prospective study design, and the relatively large sample size of patients with diabetes insipidus and primary polydipsia. We believe that our results can be generalised to the broader population.

In summary, we show that arginine infusion is a potent stimulus of the posterior pituitary. Measurements of arginine-stimulated copeptin discriminate between patients with central diabetes insipidus and those with primary polydipsia with high diagnostic accuracy. Therefore, we propose arginine stimulation as a simplified, novel, and safe diagnostic approach to diabetes insipidus.

Contributors

BW edited the protocol; contributed to data collection, data analysis, and data interpretation; did the literature search; and wrote the manuscript. NC-N wrote the protocol, contributed to data collection, and edited the manuscript. JR and COS contributed to data collection, data analysis and data interpretation, and edited the manuscript. DRV did all statistical analyses and edited the manuscript. CI, BM, MP, MS, IC, and MF contributed to data collection and edited the manuscript. GS edited the protocol, contributed to data collection, and edited the manuscript. MC-C edited the protocol, contributed to data analysis and data interpretation, edited the manuscript, and supervised all steps of the conduct of the study.

Declaration of interests

We declare no competing interests.

Data sharing

We may share de-identified, individual participant-level data that underlie the results reported in this Article and related documents, including the study protocol and the statistical analysis plan. Data will be available with the publication of our main manuscript on receipt of a request detailing the study hypothesis and statistical analysis plan. All requests should be sent to the corresponding author. The steering committee of this study will discuss all requests and decide on the basis of the scientific rigor of the proposal whether data sharing is appropriate. All applicants are asked to sign a data access agreement.

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