The water deprivation test and a potential role for the arginine vasopressin precursor copeptin to differentiate diabetes insipidus from primary polydipsia

Endocrine CONNECTIONS

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Abstract

The water deprivation test is the gold standard test to differentiate central or nephrogenic diabetes insipidus (DI) from primary polydipsia (PP) in patients with polyuria and polydipsia. Few studies have addressed the diagnostic performance of this test. The aim of this retrospective cohort study was to evaluate the diagnostic performance of the standard water deprivation test, including plasma arginine vasopressin (AVP) measurements, in 40 consecutive patients with polyuria. We compared initial test results with the final clinical diagnosis, i.e., no DI, central DI, or nephrogenic DI. The median length of follow-up was 8 years. In a subset of ten patients, the novel marker copeptin (CP) was measured in plasma. Using the final diagnosis as a gold standard, a threshold for urine osmolality of >800 mOsmol/kg after water deprivation yielded a sensitivity and specificity of 96 and 100%, respectively, for diagnosing PP. Sensitivity increased to 100% if the cut-off value for urine osmolality was set at 680 mOsmol/kg. Plasma AVP levels did not differ between patient groups and did not differentiate among central DI, nephrogenic DI, or PP. In all three patients with central DI, plasma CP was <2.5 pmol/l with plasma osmolality >290 mOsmol/kg, and >2.5 pmol/l in patients without DI. The optimal cut-off value for differentiating PP from DI during a water deprivation test was urine osmolality >680 mOsmol/kg. Differentiating between central and nephrogenic DI should be based on clinical judgment as AVP levels did not discriminate.

Key Words
- ▶ copeptin
- ▶ diabetes insipidus
- ▶ pituitary
- ▶ polydipsia
- ▶ polyuria
- ▶ primary polydipsia
- ▶ arginine vasopressin
- ▶ water deprivation test
- ▶ psychogenic polydipsia

Introduction

Polyuria (diuresis >3 l/day) in the absence of common causes, such as hypercalcemia, hyperglycemia, or relief of urinary tract obstruction, can be caused by diabetes insipidus (DI) or primary polydipsia (PP, also referred to as psychogenic polydipsia). DI can be divided in central DI, caused by impaired secretion of the antidiuretic hormone arginine vasopressin (AVP) from the posterior pituitary and nephrogenic DI, which is due to decreased sensitivity of the kidney to AVP. In DI, the primary defect is polyuria, leading to a compensatory polydipsia.

In contrast, the primary defect in PP is excessive water intake often associated with behavioral abnormalities.

DI and PP can be differentiated by performing a water deprivation test (1). In healthy subjects, water deprivation causes the plasma osmolality to rise above 280–290 mOsmol/kg, which leads to the release of AVP into the circulation. In the collecting ducts of the kidney, AVP binds to the vasopressin type 2 receptor and this is followed by expression of aquaporin 2 channels. This results in increased water retention with a rise in urine osmolality to a maximum of 1000–1200 mOsmol/kg and restoration of plasma osmolality toward the reference range (2). In DI, polyuria continues despite water deprivation, with dilution of urine in the presence of high plasma osmolality. Traditionally, the plasma AVP concentration is then used to differentiate central from nephrogenic DI. Plasma AVP should be inappropriately low in central DI and elevated in nephrogenic DI, reflecting inappropriate AVP action. In addition, the administration of the AVP analog desmopressin acetate at the end of the test may help to differentiate central from nephrogenic DI, as the antidiuretic response is theoretically preserved in central DI, but not in nephrogenic DI.

The diagnostic value of the water deprivation test has not been studied extensively. A recent review identified only 15 publications on the differential diagnosis of DI (3). These papers reported on mainly small series, used different protocols, and focused only on one part of the differential diagnostic spectrum of DI. In addition, data on the diagnostic value of AVP determination were inconsistent, probably due to its pre-analytical and analytical difficulties including a short half-life and pre-analytical instability (4, 5).

Recently, the AVP precursor copeptin (CP), which is co-secreted with AVP from the posterior pituitary, has been suggested to hold promise as a diagnostic tool in the differential diagnosis of DI, but only limited data are available (6, 7).

The aim of this study was to evaluate the diagnostic performance of the standard water deprivation test, including plasma AVP measurement, in a series of consecutive patients with polyuria in our hospital. In a small subset of cases, we explored whether CP is useful in differentiating among PP, nephrogenic, and central DI.

Materials and methods

Study population

All adult patients who underwent a water deprivation test at the Academic Medical Centre (AMC) of the University of Amsterdam between 1994 and 2011 were included. Hypercalcemia and hyperglycemia were excluded in all subjects. None of the subjects used desmopressin (DDAVP) or drugs that may interfere with the results of the water deprivation test (8, 9). Our gold standard was the final (or – if applicable – final pre-mortem) clinical diagnosis, which was determined from patient files or by contacting the patients or their treating physician (internists or general practitioner). Patients were categorized as having a clinical diagnosis of no DI, central DI, or nephrogenic DI, based on whether or not they suffered from polyuria or used desmopressin.

Water deprivation test

Patients were admitted to our ward in the evening before the test day, and fluid intake was stopped at 0700 h. Access to water was prevented by shutting down water pipes and by removing all objects containing water, such as vases. Every hour, patients were weighed and urine and blood samples were collected for the determination of plasma and urine sodium, potassium, and osmolality and of plasma AVP.

There was no pre-set maximum length of water deprivation. Water deprivation was discontinued when one of the following criteria was met: i) an urine osmolality of at least 800 mOsmol/kg. This was considered to be diagnostic of PP and ii) a weight loss of more than 5% from baseline or a plasma osmolality exceeding 300 mOsmol/kg. This was considered to be diagnostic of DI. Subsequently, if patients were known to have a pituitary disorder, a diagnosis of central DI was made. If the subject used lithium, then diagnosis of nephrogenic DI was made. If the subject neither had pituitary disease nor used lithium, we differentiated between central DI and nephrogenic DI according to the normogram for plasma AVP vs plasma osmolality or urine osmolality according to Robertson (10). In eight patients who met criterion 2, desmopressin (DDAVP) 2 µg i.v. was given and urine osmolality was measured after 1 h. From ten patients, frozen plasma samples were available to determine CP.

Laboratory analysis

Until 2005, AVP was measured with an in-house developed RIA after solid-phase extraction and standard addition to each sample. The detection limit was 0.1 pmol/l with an inter-assay variation of 8.5% at 2 pmol/l. From 2006 onwards, AVP was measured using a commercial RIA (Euria-Vasopressin, Euro-Diagnostica AB,

Table 1 Descriptives and outcomes of the water deprivation test of the study population, given as median (range).

	No DI	Central DI	Nephrogenic DI	P value no DI vs central DI
n	27	12	1	
Age (years)	33 (19–68)	46 (24–74)	51	
Nadir urinary osmolality (mOsmol/kg)	449 (64–960)	149 (56–385)	196	<0.001
Peak urinary osmolality (mOsmol/kg)	819 (680–960)	342 (67–559)	203	<0.001
Nadir plasma osmolality (mOsmol/kg)	286 (273–297)	290 (260–298)	293	0.45
Peak plasma osmolality (mOsmol/kg)	292 (276–305)	302 (296–306)	314	<0.001
Weight loss (%)	0.7 (0–4.1)	2.6 (0.8–5.4)		0.023
Minimum diuresis/h (ml)	21 (0–300)	100 (0–250)		0.007
Maximum diuresis/h (ml)	103 (18–800)	250 (130–600)		0.043
Duration of water deprivation (h)	10 (1–29)	7 (1–18)	1	0.33
AVP (pmol/l)	0.8 (0.1–4.0)	0.5 (0.1–4.3)	11.0	0.5
CP (pmol/l)	7.0 (2.0–8.8)	2.2 (1.9–2.5)		0.005

DI, diabetes insipidus; AVP, arginine vasopressin; CP, copeptin.

Malmö, Sweden), which contains the same antibody as the in-house assay. The detection limit was 0.5 pmol/l and the inter-assay variation 11.5% at 2.5 pmol/l. CP was measured by an immunoluminometric assay (BRAHMS CT-proAVP LIA, BRAHMS GmbH, Thermo Scientific, Henningsdorf, Germany). The detection limit was 0.4 pmol/l and inter-assay variation 12.4% at 4 pmol/l. Osmolality was measured on OSMOstation OM-6050 (Arkray, Kyoto, Japan).

Statistical analysis

Data were analyzed using the SPSS Software (IBM, version 20). Results are expressed as median and range. Differences between patient groups were assessed using the Mann–Whitney *U* test. A *P* value <0.05 was considered statistically significant.

Results

Patients

A total of 70 water deprivation tests were performed between 1994 and 2011. Sixteen tests were stopped for unknown reasons while formal stopping criteria were not. Four could not be evaluated because of missing data. Ten patients were lost to follow-up. This resulted in 40 water deprivation tests in 12 men and 28 women who had undergone a complete standard water deprivation test and for whom a final clinical diagnosis was established (no DI, central DI, or nephrogenic DI) (Table 1). The median length of follow-up was 8 years (range 2–18 years).

Out of 40 patients, 15 had pituitary disease (*n*=1 sarcoidosis, *n*=1 Langerhans cell histiocytosis, *n*=1 Rathke's cleft cyst, *n*=7 transsphenoidal resection of a

sellar tumor (either with or without radiotherapy), *n*=4 pituitary insufficiency after radiotherapy for a cerebral tumor, and *n*=1 Sheehan syndrome). Two patients had polyuria after a head trauma. One patient had polyuria while using lithium. The remaining 22 patients had unexplained polyuria.

Final clinical diagnosis

The final clinical diagnosis was no DI in 27 patients, central DI in 12 patients, and nephrogenic DI in one patient. The patient with nephrogenic DI used lithium. Of the patients with central DI, seven had pituitary disease, one had suffered a head trauma, and four were diagnosed according to the normogram. All patients with DI still used desmopressin or had used desmopressin until they died.

In all but two patients, the final clinical diagnosis was identical to the original diagnosis based on the water deprivation test. The two exceptions were patient no. 1, who developed polyuria after a head trauma. After 16 h of water deprivation, the test was terminated because of a plasma osmolality of 306 mOsmol/kg and

Table 2 Two-by-two table based on the current cut-off value for urine osmolality (>800 mOsmol/kg).

	Final clinical diagnosis		
	DI	No DI	Total
First criterion reached			
Maximum urine osmolality >800 mOsmol/kg	0	26	26
Plasma osmolality >300 mOsmol/kg or weight loss >5%	13	1	14
Total	13	27	40

DI, diabetes insipidus.

Table 3 Two-by-two table based on a cut-off value for urine osmolality of >680 mOsmol/kg.

	Clinical diagnosis		
	DI	No DI	Total
First criterion reached			
Maximum urine osmolality >680 mOsmol/kg	0	27	27
Plasma osmolality >300 mOsmol/kg or weight loss >5%	13	0	13
Total	13	27	40

DI, diabetes insipidus.

a urine osmolality of 480 mOsmol/kg. The patient subsequently used desmopressin for several months. After cessation of desmopressin; however, polyuria did not recur. Temporary DI is a well-known complication of severe head trauma (11). Therefore, we consider this patient to have had a temporary central DI. Patient no. 2 suffered from Wegener's disease and concomitant polyuria. She had no pituitary, neurological disease or head trauma. After 22 h of water deprivation, she had a plasma osmolality of 301 mOsmol/kg and a urine osmolality of 680 mOsmol/kg. At this point, her diuresis had decreased to 0 ml/h. Based on the test criteria, the diagnosis was DI. However, the anuria during water deprivation made us conclude that she did not suffer from DI. As anticipated, the polyuria disappeared spontaneously within a year after the test. She had not used desmopressin at any time.

Test criteria

Test parameters of both patient groups are given in Table 1. In 26 patients, the first criterion that was reached was a urine osmolality >800 mOsmol/kg (after a median of 10 h, range 1–29 h), indicative of PP. All these patients have a final clinical diagnosis of PP (sensitivity 96%, specificity 100%, Tables 2 and 3). One of the patients who did not reach a urine osmolality of 800 mOsm/kg had a final clinical diagnosis of PP (patient no. 2). The remaining 14 patients continued the water deprivation until the plasma osmolality exceeded 300 mOsmol/kg (12 patients) or weight loss exceeded 5% (two patients). Seven of these patients, all with a final clinical diagnosis of central DI, received desmopressin at the end of the test with a median rise in urine osmolality of 75 mOsmol/kg (range 41–248 mOsmol/kg).

The median duration of water deprivation was 7 h (range 1–18 h). Sensitivity and specificity for DI or PP

increased to 100% and 100% if the cut-off value for urine osmolality was set at 680 mOsmol/kg.
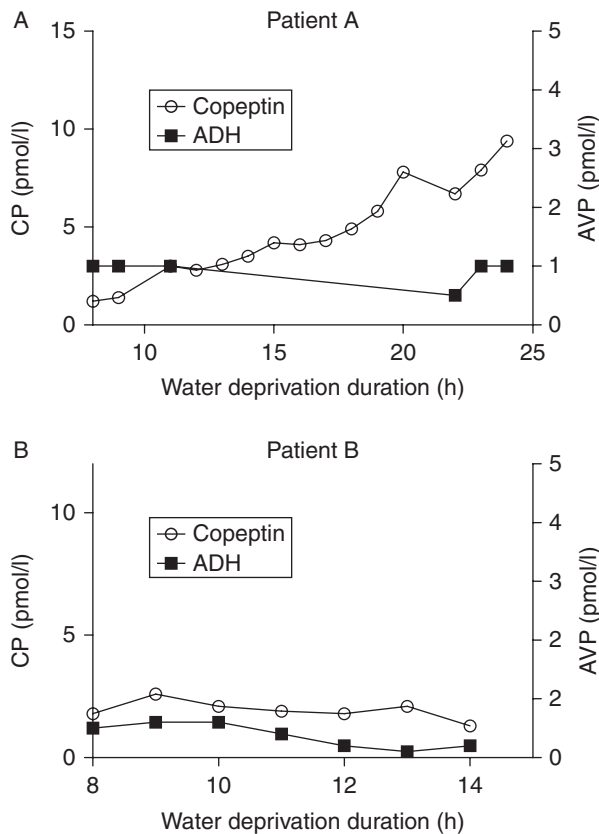
AVP and CP

Plasma AVP levels at the time of discontinuation of the test showed considerable overlap between patient groups (no DI: 0.8 pmol/l (0.1–4.0 pmol/l), central DI: 0.5 pmol/l (0.1–4.3 pmol/l) ($P=0.5$), nephrogenic DI 11.0 pmol/l) (Fig. 1).

As shown in Fig. 1, plasma CP from the ten patients at discontinuation of the test was significantly lower in patients with DI compared with those without DI (central DI 2.2 pmol/l (1.9–2.5 pmol/l) vs no DI 7.0 pmol/l (2.0–8.8 pmol/l); median (range), $P=0.005$). When taking into account only samples with a plasma osmolality >290 mOsmol/kg, CP levels in patients with central DI
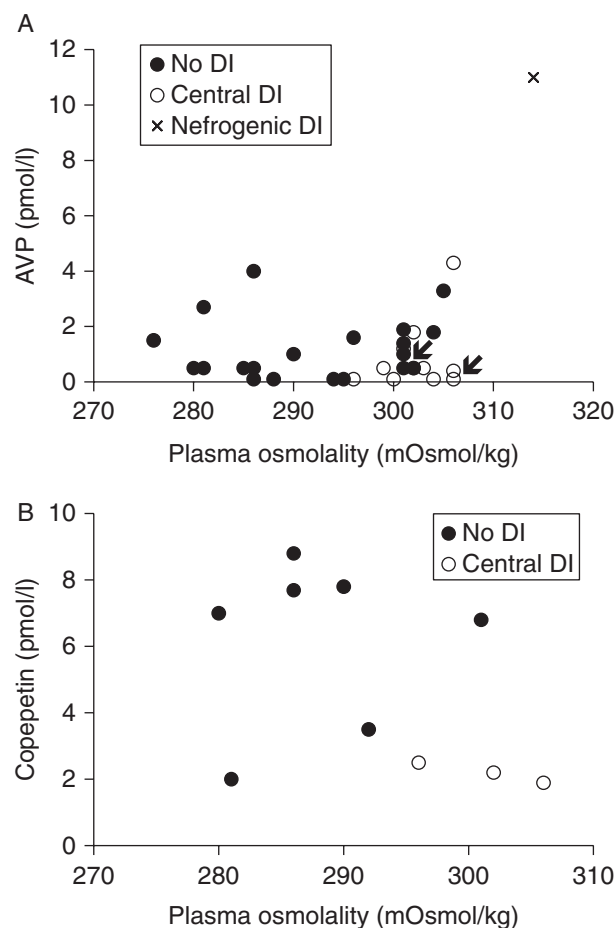
Figure 1
Plasma osmolality vs AVP (A) and CP (B) at the end of the water deprivation test. Arrows depict patient 1 (in the central DI group) and patient 2 (in the no DI group). DI, diabetes insipidus; CP, copeptin.

Figure 2
AVP and CP during water deprivation in a subject with PP (A) and a subject with central DI (B). DI, diabetes insipidus; AVP, arginine vasopressin; CP, copeptin.

were <2.5 pmol/l, but none was in the group without DI (P<0.0001).

In two patients, hourly CP and AVP data were available (Fig. 2). Patient A did not have DI, and CP levels clearly increased during the test, while AVP levels remained low (Fig. 2). In patient B, who had central DI, both AVP and CP plasma levels remained low (Fig. 2).

Discussion

In this study, we evaluated the diagnostic performance of the water deprivation test as adapted from Miller *et al.* (1) in a series of consecutive patients to differentiate among PP, central, and nephrogenic DI. We also report on the novel marker CP in a small subset of patients.

In our series, the best parameter for diagnosing PP was a urine osmolality of >680 mOsmol/kg after water deprivation (sensitivity and specificity 100%). The original cut-off value of 800 mOsmol/kg resulted in a lower accuracy, misdiagnosing one patient as having DI.

Patients who did not achieve a urine osmolality of >680 mOsmol/kg, but instead reached a plasma osmolality >300 mOsmol/kg or experienced weight loss of >5%, all had DI.

AVP levels did not differ between the patient groups. CP seemed to be a more promising marker. In our limited dataset for CP, all patients with central DI had a CP level below 2.5 pmol/l at supranormal osmolality (>290 mOsmol/kg).

Importantly, we found that plasma AVP is not useful in the establishment of a final diagnosis. This may be due, at least in part, to methodological issues measuring plasma AVP. First, there is marked pre-analytical instability (5). In our study, AVP was collected in EDTA tubes with 500 kIU aprotinin/ml blood, and kept at 0–4 °C during transport and centrifugation. Samples were stored at −20 °C for a maximum of 2 months until assay. Therefore, it is unlikely that proteolytic degradation is responsible for the low values of AVP. By extracting AVP with a solid phase, some loss may occur, hence, in both RIAs, recovery was measured and corrected for. Furthermore, antibody heterogeneity between different immunoassays can give variable results. However, both assays in this study used the same antibody and this antibody has no cross-reactivity with oxytocin (12). Therefore, we cannot explain the poor diagnostic performance of plasma AVP in our series.

Recently, CP has been proposed as a surrogate for AVP. CP is the C-terminal part of the AVP precursor that is co-secreted with AVP from the neurohypophysis. CP has a high *in vitro* stability and is relatively easy to measure (7). In a recent study, CP levels before start of water deprivation could be used to identify patients with complete central or nephrogenic DI (6). Unfortunately, our patient set in whom plasma CP levels were measured was small, but the preliminary results do hold promise in this respect.

There were several differences between our study and earlier studies. First, many authors have used hypertonic saline infusion instead of water deprivation to raise plasma osmolality (3). Others, such as the recent study by Fenske *et al.* (6) used water deprivation, but for a fixed period of 16 h during which only a limited number of samples were taken. The length of fluid restriction in our protocol depends on weight loss, and plasma- and urine osmolality, which is measured every hour. This makes our test more labor intensive and implies that for some subjects, particularly those with PP, the duration of the test is longer. However, for subjects with DI, who are at a serious risk for dehydration, the test can be terminated earlier.

Another difference is that most studies used the relationship between plasma and urine osmolality as well as the relative increase in urine osmolality to differentiate DI from PP. We used absolute cut-off values for plasma and urine osmolality, which gave an excellent accuracy. Finally, our gold standard was the current clinical diagnosis of the patient. We feel that in the absence of a validated gold standard for diagnosing DI, this is the most appropriate approach.

One of the limitations of our study is that we did not systematically evaluate the response to desmopressin. Furthermore, a relatively large number of tests could not be used because of missing data or a loss to follow-up. In addition, we made no differentiation between complete and partial DI, thus potentially missing some of the mild cases of DI. The partial forms of DI are especially difficult to distinguish from chronic PP for several reasons. First, in patients with chronic PP aquaporin 2 channels in the kidney are down-regulated and there is washout of the renal medullary concentration gradient, resulting in inappropriately dilute urine and a poor response to exogenous AVP (13). This situation mimics DI. Secondly, patients with partial forms of DI may be able to concentrate urine to levels above plasma osmolality during dehydration (10, 14).

Based on our study, we conclude that the best parameter for differentiating PP from DI is urine osmolality, which is diagnostic for PP when exceeding 680 mOsmol/kg after prolonged water deprivation. Our data show that plasma AVP does not help to make this distinction. As suggested by others, we found that plasma CP holds promise in this respect. However, its role should be studied more extensively in larger groups and in a prospective manner.

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