DIAGNOSIS OF ENDOCRINE DISEASE

The role of the desmopressin test in the diagnosis and follow-up of Cushing’s syndrome

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Abstract

Desmopressin is a vasopressin analogue selective for type 2 vasopressin receptors that mediate renal water retention. In contrast to the native hormone arginine vasopressin, a well-known ACTH secretagogue, desmopressin, exerts minimal or no activity on ACTH excretion. However, in a substantial proportion of patients with ACTH-dependent Cushing’s syndrome (CS), desmopressin elicits an ACTH and cortisol response, which contrasts with the minimal responses obtained in healthy subjects. The mechanism underlying this paradoxical response involves upregulation of vasopressin type 3 and/or the aberrant expression of type 2 receptors by neoplastic ACTH-producing cells. This makes desmopressin administration a suitable test enabling the distinction between neoplastic from functional (formerly termed ‘pseudo-Cushing syndrome’) ACTH-dependent cortisol excess. Several studies have now established an adjunctive role of desmopressin in the initial diagnostic workup of CS. Despite some early data indicating that this test may also have a role in distinguishing between Cushing’s disease (CD) and ectopic ACTH secretion, subsequent studies failed to confirm this observation. The ability of the paradoxical response to desmopressin to depict the presence of neoplastic ACTH-secreting cells was also exploited in the follow-up of patients with CD undergoing surgery. Loss of the desmopressin response, performed in the early postoperative period, was a good predictor for a favorable long-term outcome. Moreover, during follow-up, reappearance of desmopressin paradoxical response was an early indicator for recurrence. In conclusion, the desmopressin test is a valid tool in both the diagnosis and follow-up of patients with CD and should be more widely applied in the workup of these patients.

Introduction

Besides its well-known antidiuretic and pressor actions, the neurohypophyseal nonapeptide arginine vasopressin (AVP) has an important role in the regulation of ACTH secretion; it is both a major direct secretagogue of ACTH and a potentiator of the ACTH-releasing activity of corticotropin-releasing hormone (CRH) \(^1, 2, 3, 4\).
In fact, it was the first hypothalamic factor that was recognized to possess ACTH-releasing properties (5, 6), long before the characterization of CRH (7). Although the discovery of CRH overshadowed initially the significance of AVP as an ACTH secretagogue, numerous studies have now established an important role of this peptide in the physiology of the hypothalmo–pituitary–adrenal (HPA) axis and of its synthetic analogues in the evaluation of patients with CS (8).

AVP acts though three distinct types of receptors. The V1 (or V1a) receptor stimulates the phosphoinositol cascade and mediates AVP’s pressor and hepatic (glycogenolysis and neoglycogenesis) effects (9, 10). Its antidiuretic action occurs through the V2 receptors in the kidney through cAMP-mediated signaling (11). Via the same receptor, AVP also mediates its hemostatic effects. The pituitary vasopressin receptor has been designated as V3 (or V1b) receptor, and it is clearly distinct from the other two receptors (12); it is not blocked by anti-pressor antagonists (13), and a vasopressin analogue, deamino[d-3-(3'-pyridyl)-Ala2, Arg8] vasopressin, possesses specific ACTH-releasing activity (14) with negligible pressor and antidiuretic effects.

In early studies, ACTH and cortisol responses to the administration of AVP, or more often LVP (lysine vasopressin, the porcine antidiuretic hormone), were evaluated in healthy subjects as well as in patients with pituitary disorders (6, 15, 16, 17, 18, 19, 20). Evaluation of vasopressin analogue administration in the diagnostic workup of Cushing’s syndrome (CS) was of special interest; LVP and AVP stimulated the secretion of ACTH and cortisol in patients with CD (17, 21, 22, 23, 24) and had a synergistic effect with CRH (24). In the discovery of CRH, however, displaced the use of LVP from the diagnostic workup of ACTH-dependent CS (25) because of its better discriminatory accuracy between CD and ectopic ACTH syndrome (EAS) (22) and less side effects compared to those of LVP (nausea, abdominal pain, flushing).

Desmopressin (1-deamino-S-arginine vasopressin, DDAVP) is a synthetic analogue of AVP that is selective for the renal V2 receptor (26) resulting in prominent antidiuretic but negligible pressor effect (antidiuretic-to-pressor ratio of 4000). It became the treatment of choice for central diabetes insipidus (27) because of its effectiveness, long duration of action and lack of pressor and oxytocin effects (28). It is also a treatment option for patients with mild hemophilia A and type I von Willebrand disease due to its ability to increase the levels of plasma von Willebrand factor, FVIII:C and tissue plasminogen activator (29), and also exerts a vasodilatory effect, as a result of its direct effect on the endothelium, via activation of the endothelial vasopressin V2 receptor and activation of the endothelial NO synthase (30). In general, administration of desmopressin is well tolerated with only minimal and transient side effects, such as small increases in blood pressure and heart rate, slight head heaviness, nausea, flushing and cold sensation (31). Water intoxication may be a risk, particularly in children and in patients with congestive heart failure on a high fluid intake and care must be taken to advise patients to restrict fluids on the day of the test. Overall, the incidence of adverse reactions is considered low, comparable to that of CRH and much less than those of LVP (32). Over the last few decades, several groups used desmopressin for the investigation of the HPA axis and more specifically for the workup of CS. It was suggested that desmopressin may have an adjunctive role in the diagnosis and follow-up of CS and, herein, we will review the published data demonstrating its clinical utility in patients with this intriguing disorder.

### Desmopressin effect on ACTH secretion in healthy subjects

At variance with an ACTH-releasing effect seen in rats (33, 34), desmopressin has no significant affinity for the human pituitary V3 receptor, despite in vitro evidence for a weak ACTH-releasing activity and a synergistic effect with CRH. In early clinical studies, desmopressin was administered at various doses in healthy human subjects. Andersson et al. (16) investigated the effect of two desmopressin doses (4 μg and 16 μg) given intravenously in 17 young male volunteers, and there were no significant increases in cortisol levels. Gaillard et al. (2) observed a slight albeit not significant rise in ACTH levels during a 2-h desmopressin infusion (1 ng/kg/min). When co-administered with ovine CRH (oCRH), desmopressin increased the response of ACTH compared to the response seen with oCRH alone, but to a much lesser extent than the sum of the responses to the two agents separately. In the study by Malerbi et al (35), administration of 10 μg of desmopressin increased cortisol levels in only 2 of the 15 subjects by 58% and 68% respectively. In the same study, a group of patients with depression was included; interestingly, they also did not exhibit significant responses to desmopressin, despite evidence of a disturbed HPA axis. In the study of Rado and Juhos (36) an average increase of 6.9 ± 1.7 μg/dL in plasma cortisol after the i.v. administration of 4 μg of desmopressin was reported in 12 of the 20 studied subjects.
The observed increase, however, was considerably lower than that observed after administration of LVP to the same subjects. In the same study, intranasal administration of desmopressin had no effect. Williams et al. (37) reported a moderate effect of desmopressin on cortisol levels, but much larger doses of desmopressin were administered (0.4μg/kg). It should be noted that the time of the day of desmopressin administration might influence its ACTH-releasing potential. In the study of Scott et al. (38), three different doses of desmopressin (5, 10 and 15μg) were administered in the early afternoon; they report a significant ACTH response to all three doses, with 10μg producing maximal responses, and a significant cortisol response to 10 and 15μg doses. Overall, 11 of the 18 subjects were deemed ‘responders’ to 10μg desmopressin on the basis of both ACTH and cortisol output. The early afternoon is a period of HPA axis quiescence with higher endogenous CRH levels compared to the morning levels. It is therefore possible that the observed effect of desmopressin administered in the afternoon reflects a CRH-mediated pituitary activation, given a few studies reporting a potentiating effect of desmopressin on exogenously administered CRH in healthy subjects (39, 40). Most of the subsequent reports failed to demonstrate appreciable responses to desmopressin in healthy subjects (2, 31, 39, 40, 41, 42, 43, 44).

To summarize, despite some variability between studies that relates to the dose, the mode of administration (continuous infusion vs bolus injection), the time of investigation and also the criteria to define a positive response, it seems that desmopressin administration has a negligible or no ACTH-releasing activity in the majority of healthy human subjects.

Desmopressin effect in patients with Cushing’s syndrome

Malerbi et al. (31) were the first to evaluate the cortisol responses to desmopressin (5 or 10μg) in patients with CS of various etiologies, including patients with ACTH-independent hypercortisolism. A cortisol increment of more than four times the intra-assay coefficient of variation at baseline concentration (corresponding to a 40–45% increase) was seen in all but one of the 16 patients with CD and in the two patients with ACTH-dependent adrenal hyperplasia (presumably representing also patients with long-standing CD), but not in the 8 patients with adrenal CS (albeit one had a response of 42% that was deemed borderline). Similar results showing enhanced ACTH and cortisol responses following the administration of desmopressin in patients with ACTH-dependent CS were reported in many subsequent studies (31, 35, 41, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55) (Tables 1 and 2).

The mechanism of such an exaggerated and thus paradoxical response to desmopressin in patients with ACTH-dependent CS is not fully elucidated. Although vasopressin induces ACTH secretion by acting directly on the pituitary V3 receptor and by potentiating the action of CRH, desmopressin does not seem to have considerable activity on the V3 receptor. Therefore, other mechanisms have to be considered. One proposed mechanism is that desmopressin, despite its low cross-affinity with the V3 (V1b) receptor may demonstrate some activity when the V3 receptor is present in high concentrations, either due to a hypercortisolism-induced upregulation (56) or because of a constitutional overexpression in corticotroph adenomas or other types of ACTH-producing tumors (57, 58, 59). Another, probably additional, mechanism is that the V2 receptor is expressed aberrantly by the abnormal ACTH-secreting cells (59); in fact, Dahia et al. reported the expression or the V2 receptor in most of the studied corticotrophin tumors (58).

The V3 receptor has been reported to also be commonly expressed in ectopic ACTH-secreting (EAS) tumors (57) and explains the fact that administration of nonspecific vasopressin analogs, such as LVP or AVP results in positive cortisol and ACTH responses in most patients with EAS. Ectopic expression of the V2 receptors in EAS tumors that are responsive to desmopressin has also been documented (55, 58, 60). We demonstrated (55) the presence of the V2R mRNA in all the four tumors that we examined, whereas the V3R mRNA was expressed in three of the four cases. Due to the limited number of studied patients with EAS, however, the frequency of positive in vivo responses to desmopressin as well as the ectopic expression of the V2R remains unclear. Whether some tumor types are more likely to respond to desmopressin compared to others, also remains elusive because the number of reported cases is small.

Despite the lack of concrete knowledge about the mechanism, a substantial proportion of patients with ACTH-dependent CS demonstrate a paradoxical ACTH and cortisol response to desmopressin and, consequently, a series of studies exploring the value of a ‘desmopressin test’ (DT) in the diagnosis and differential diagnosis of ACTH-dependent CS were conducted. To this end, a fixed dose of 10μg of desmopressin given i.v. was used in most studies.
Table 1  The desmopressin stimulation in patients with Cushing's disease (CD), suspected Cushing's syndrome (CS) or pseudo-Cushing's syndrome (PCS) and healthy subjects.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients with CD, R/TN (%)</th>
<th>Suspected CS/PCS</th>
<th>Healthy subjects, R/TN (%)</th>
<th>Criteria</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(31)</td>
<td>15/16 (94%)</td>
<td>4/11 (36%)</td>
<td>2/15 (13%)</td>
<td>%ΔCort &gt;40–45%</td>
<td>Sensitivity (%) 94  Specificity (%) 87</td>
</tr>
<tr>
<td>(35)</td>
<td>14/14 (100%)</td>
<td>Depression</td>
<td>2/20 (10%)</td>
<td>%ΔCort &gt;36%</td>
<td>100 vs controls 64 vs depressed 100</td>
</tr>
<tr>
<td>(41)</td>
<td>13/16 (81%)</td>
<td>0/4 (0%)</td>
<td>0/15 (7%)</td>
<td>%ΔCort &gt;20% and %ΔACTH &gt;50%</td>
<td>81</td>
</tr>
<tr>
<td>(43)</td>
<td>10/10 (100%)</td>
<td>0/11 (0%)</td>
<td>%ΔACTH &gt;150%</td>
<td>100 vs depressed 100</td>
<td></td>
</tr>
<tr>
<td>(44)</td>
<td>21/25 (84%)</td>
<td>3/20 obese (15%)</td>
<td>%ΔCort &gt;20%</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>(47)</td>
<td>66/76 (87%)</td>
<td>1/30 (3%)</td>
<td>%ΔACTH &gt;50%</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>(52)</td>
<td>17/19 (90%)</td>
<td>Alcohol dependence, depression, PCOS</td>
<td>5/31 normal (16%) 3/36 obese (8%)</td>
<td>ΔACTH &gt;27 pg/mL</td>
<td>87</td>
</tr>
<tr>
<td>(49)</td>
<td>22/27 (82%)</td>
<td>2/23 (7%)</td>
<td>Central obesity, PCOS, depression, alcohol dependence</td>
<td>–</td>
<td>82</td>
</tr>
<tr>
<td>(46)</td>
<td>13/15 (87%)</td>
<td>0/15 (0%)</td>
<td>%ΔACTH &gt;35% and ΔACTH &gt;20 pg/mL</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>(53)</td>
<td>NR/52</td>
<td>NR/28</td>
<td>ΔACTH &gt;27 pg/mL</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>(54)</td>
<td>NR/30</td>
<td>NR/18</td>
<td>cortisol &gt;12 μg/dL and ΔACTH &gt;18 pg/mL</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>(50)</td>
<td>NR/68</td>
<td>NR/56</td>
<td>cortisol &gt;12 μg/dL and ΔACTH &gt;18 pg/mL</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

R/TN(%), responders/total number tested (%); CD, Cushing's disease; CS, Cushing's syndrome; PCS, pseudo-Cushing's syndrome; PCOS, polycystic ovaries syndrome; NR, not reported; %ΔCort, ((peak cortisol-basal cortisol)/basal cortisol)×100%; %ΔACTH, ((peak ACTH-basal ACTH)/basal ACTH)×100%; ΔACTH, peak ACTH-basal ACTH; B cortisol, baseline cortisol.
The desmopressin test in the diagnosis of Cushing’s syndrome

The lack of response to desmopressin in subjects without ACTH-dependent hypercortisolism, makes it a suitable test for the diagnosis of CS. In fact, the pathophysiological basis of the response to desmopressin is distinct from that of the commonly used screening tests, which assess functional alterations of the HPA axis. The desmopressin response represents an aberrant response, mostly depicting neoplastic cells and thus it is particularly useful especially in situations where the discrimination between functional and neoplastic ACTH-dependent hypercortisolism is ill defined, such as in patients with the so-called ‘pseudo-CS’ (PCS). The term ‘pseudo-Cushing’ is probably a misnomer and has a broad definition. It has been used to characterize conditions that affect the specificity of the classic diagnostic tests, because they cause a state of chronic hypercortisolism due to ‘physiologic’ HPA axis activation (61). This is the case in conditions such as alcoholism, depression, chronic kidney disease or uncontrolled diabetes mellitus. It also refers to patients with several clinical features resembling CS like central obesity, hypertension, hirsutism, irregular menses, but with hormonal testing that is not clearly diagnostic. A major challenge is to differentiate these groups from ‘true’ or neoplastic, ACTH-dependent hypercortisolism.

Thus, besides the evaluation of the DT in healthy subjects, of special interest is the performance of this test particularly in populations who are more likely to be subjected to testing for the presence of CS. Studies assessing the diagnostic performance of the DT and the proposed cut-offs are presented in Table 1. It must be noted that all studies were conducted in patients with ACTH-dependent CS and in particular in patients with CD, whereas no patients with EAS were included. This, however, is more relevant to the clinical challenges since it is usually CD, and especially the milder cases, that bear difficulties in distinguishing them from non-neoplastic hypercortisolism, whereas patients with full-blown CS do not usually pose diagnostic problems. Two studies (49, 53) performed a separate analysis of patients with mild CD, defined as having at least one normal or borderline abnormal biochemical test and showed a similar sensitivity of the DT to that when the whole cohort was considered. Of note in the study by Tirabassi et al. (53), the DT distinguished mild CD from PCS more accurately than did the overnight dexamethasone suppression and midnight serum cortisol tests.

Currently, the dexamethasone-suppressed CRH (Dexa-CRH) test is widely used for the distinction between CD and PCS. Pecori Giraldi et al. compared retrospectively the diagnostic ability of the DT with this test in 32 patients with CS and 23 with PCS (49). Of note, all but 2 patients with CD already exceeded the diagnostic cut-off of the Dexa-CRH test after the dexamethasone suppression alone, so that administration of CRH was of no additional benefit. At the same time, a substantial number of PCS subjects had a positive Dexa-CRH test, due to either non-suppression of cortisol after dexamethasone or due to cortisol increases that were minor but still sufficient to exceed the threshold of 38 nmol/L (1.4 μg/dL), resulting in a false-positive rate of 21% and a specificity of 62.5%. On the other hand, although some CD patients were non-responders to the DT, resulting in a sensitivity of 81.5%, the rate of false positives was substantially lower leading to a specificity of 90%. Similar observations were obtained in a subgroup of patients with mild CD, defined as having UFC <690 nmol/24-h (250 μg/24-h). Raising the threshold for the Dexa-CRH test to 110 nmol/L (4 μg/dL) improved its specificity to 82% but at the expense of sensitivity. However, Bayesian analysis, performed in the same study (49), showed that the best diagnostic outcome was obtained by combining the dexamethasone suppression test (DST) and the DT, whereas the combination of DST with Dexa-CRH tests was statistically inferior. This is attributable to the fact that the former panel combines a test of high sensitivity with a test with high specificity while the latter panel is compromised.

Table 2 The desmopressin stimulation in the differential diagnosis of ACTH-dependent Cushing’s syndrome.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients with CD</th>
<th>Patients with EAS</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>(31)</td>
<td>15/16 (94%)</td>
<td>0/1</td>
<td>%ΔCort &gt;40–45%</td>
</tr>
<tr>
<td>(42)</td>
<td>14/17 (82%)</td>
<td>3/4 (75%)</td>
<td>%ΔACTH &gt;20% and %ΔCort &gt;20%</td>
</tr>
<tr>
<td>(41)</td>
<td>13/16 (81%)</td>
<td>0/1</td>
<td>%ΔCort &gt;20% and %ΔACTH &gt;35%</td>
</tr>
<tr>
<td>(43)</td>
<td>10/10 (100%)</td>
<td>0/3</td>
<td>%ΔACTH &gt;150%</td>
</tr>
<tr>
<td>(44)</td>
<td>23/25 (92%)</td>
<td>0/3</td>
<td>%ΔACTH &gt;50% and/or %ΔCort &gt;20%</td>
</tr>
<tr>
<td>(52)</td>
<td>17/19 (89%)</td>
<td>2/5 (40%)</td>
<td>%ΔCort &gt;50% and/or %ΔACTH &gt;20 pg/mL</td>
</tr>
<tr>
<td>(55)</td>
<td>19/26 (73%)</td>
<td>3/5 (60%)</td>
<td>%ΔACTH &gt;35% and %ΔCort &gt;20%</td>
</tr>
<tr>
<td>(51)</td>
<td>19/22 (86%)</td>
<td>4/9 (44%)</td>
<td>%ΔACTH &gt;50%</td>
</tr>
<tr>
<td>(45)</td>
<td>NR/149 (sensitivity 83%)</td>
<td>NR/21 (specificity 62%)</td>
<td>%ΔACTH &gt;32%</td>
</tr>
</tbody>
</table>

CD, Cushing’s disease; EAS, ectopic ACTH syndrome; NR, not reported; %ΔCort, (peak cortisol-basal cortisol)/basal cortisol) × 100%; %ΔACTH, ((peak ACTH-basal ACTH)/basal ACTH) × 100%.
by the low specificity of the Dexe-CRH test. In fact, the specificity of the Dexe-CRH is also hampered in patients with incidentally discovered bilateral adrenal nodules; we recently reported that a significant number of such patients respond to this test (62). In contrast with patients with clinically overt adrenal CS, many of these patients do not have fully suppressed baseline ACTH levels and the use of the Dexe-CRH test may lead to the erroneous diagnosis of ACTH-dependent CS. In contrast, none of the responders tested positive to the DT. In addition, it is well established that the specificity of the DST is affected by the use of medications that interfere with the dexamethasone metabolism (63), whereas the response to the DT is not affected, making the desmopressin test a plausible choice for these patients.

Overall, the DT has a good diagnostic performance, even where the first-line tests have a high rate of false-positive (like in PCS) or false-negative (mild CD) results and thus it may represent a good second-line test especially in these situations. The DT can be particularly useful in patients with a low clinical suspicion but non-suppressed cortisol levels after the low-dose DST, where the Dexe-CRH is ‘a priori’ positive.

The desmopressin test in the differential diagnosis of ACTH-dependent Cushing’s syndrome

In the study by Malerbi et al. (31) the only patient with proven and two patients with suspected EAS that were included were unresponsive. This observation initially suggested that a positive response to desmopressin might indicate CD, in analogy to the response to CRH, and led to studies aiming to evaluate the role of desmopressin in the differential diagnosis of ACTH-dependent CS. Colombo et al. (41) demonstrated a positive cortisol and ACTH response in 14 of 17 patients with CD but not in the single patient with suspected EAS. Sakai et al. (43) also reported that none of the 3 patients with EAS responded to desmopressin, whereas all 10 patients with CD did; they applied, however, a fairly high threshold for the percent ACTH rise of 120%. Subsequent studies, however, identified an increasing number of positive results in patients with proven EAS (Table 2). Newell Price et al. (48) compared the discriminatory ability of the desmopressin to that of the CRH test and, also, to a combined test using both agents in patients with ACTH-dependent CS (17 patients with CD and 5 patients with EAS). One patient with EAS had a positive cortisol response and 3 positive ACTH responses to the DT resulting in lower discriminating accuracy of the DT compared to the CRH test. Positive responses to desmopressin were also reported by Terzolo et al. (52) in 2 out of 5 patients with EAS and in case reports (60). Our results in a cohort of 29 patients with CD and five with EAS (55), also indicated that a proportion of patients with EAS respond to the DT; we observed positive responses in 3 of the 5 patients with histologically confirmed EAS and found a significant overlap of the percent cortisol and ACTH responses to the desmopressin test between patients with CD and EAS. In fact, ROC curve analysis showed that there was no good criterion that can distinguish between the two groups of CD and EAS patients.

Another intriguing hypothesis was that combining desmopressin with CRH may improve the discriminatory ability of both tests. In contrast to EAS tumors, corticotroph adenomas commonly express the CRH receptor (57, 59). Based on the hypothesis that co-overexpression of the CRH and V3 (V1b) or V2 receptors in many corticotroph adenomas but not in tumors secreting ACTH ectopically, Newell Price et al. (42) combined desmopressin with CRH. They observed greater percentage increases of cortisol and ACTH, compared to each compound alone, providing evidence for a considerable CRH-potentiating action of desmopressin on ACTH release in patients with ACTH-dependent CS, as opposed to what was previously reported in healthy subjects (2). Moreover, the combination of desmopressin and CRH appeared to yield better results for the differential diagnosis of CD from EAS, than either peptide alone. In our cohort (55), the combined desmopressin-CRH test performed better than DT alone, when the ACTH (but not cortisol) percentage increment is considered, but still with only a moderate performance, regarding the differential diagnosis between CD and EAS.

Overall, the available data do not support a role of the DT in the differential diagnosis of ACTH-dependent CS. The utility of the combined desmopressin-CRH test as an adjunctive test to the CRH test, especially in patients with negative CRH test, is limited.

Bilateral Inferior Petrosal Sinus Sampling (BIPSS)

It is well established that BIPSS represents the most accurate diagnostic procedure for the differential diagnosis between pituitary-derived and ectopic ACTH hypersecretion (64). However, the basal ACTH gradient between the central and peripheral samples is not always diagnostic because of intermittent ACTH secretion from the neoplastic corticotrophs and therefore sampling during an ACTH nadir may result in a false-negative ratio...
Stimulation of ACTH secretion with CRH improves the diagnostic sensitivity of this test and is part of the standard BIPSS procedure (65, 66, 67).

Desmopressin has also been used as an alternative ACTH stimulant during BIPSS. A few studies used desmopressin instead of CRH during BIPSS (68, 69, 70) and reported a sensitivity comparable to that reported for CRH (92.1–97%), with an excellent specificity. The number, however, of studied subjects is small, especially when compared to the number of subjects studied with the use of CRH stimulation.

Although BIPSS is a highly accurate test, its sensitivity in the various studies ranges from 88 to 100%, owing to a low but still considerable rate of false-negative results (71, 72, 73). One reason for a false-negative gradient is poor responsiveness of some corticotroph adenomas to CRH, which involves around 10–20% of patients with CD (74, 75). On the basis of the synergistic effect of desmopressin and CRH on the release of ACTH in patients with CD (42), it was hypothesized that the administration of both these agents during BIPSS may improve its sensitivity. In this context, Kaltzas et al. (76) reported a higher ACTH output from pituitary corticotroph adenomas after the combined administration of desmopressin and CRH during BIPSS in 6 patients with CD. In a larger number of patients (77), we confirmed that the combined desmopressin/CRH stimulus results in higher central ACTH levels compared to the levels observed in patients who underwent BIPSS with CRH stimulation alone. We subsequently demonstrated, in a study of 54 patients (78), including 7 patients with histologically proven EAS, that this amplified stimulation improved the sensitivity of the procedure to 98% (compared to 62% for the basal central-to-peripheral ACTH gradients) without compromising its specificity, which was 100%.

All 8 patients with CD who were unresponsive to CRH had diagnostic central-to-peripheral ACTH gradients and, importantly, when only the subgroup of 18 patients (16 with CD and 2 with EAS) with contradictory results to tests with CRH and/or high-dose DST were separately analyzed, the sensitivity, specificity and accuracy of BIPSS were 100%.

Altogether, the available data support that desmopressin may be an equally effective and less expensive alternative ACTH stimulant during BIPSS, especially when CRH is unavailable (79). Moreover, the combined administration of desmopressin with CRH may improve the diagnostic sensitivity of BIPSS, especially in patients with negative results to the routine non-invasive differential diagnostic tests and in particular to CRH testing. So far, there is no evidence that a more potent stimulus might result in the loss of specificity, but certainly, more data on patients with EAS are required. Although the administered desmopressin dose during BIPSS is roughly half the hemostatic dose (80), considering that CS is associated with hypercoagulation (81), and that BIPSS is a procedure associated with a minor but not negligible risk of thromboembolism (82), it is advised to routinely heparinize patients undergoing BIPSS with desmopressin stimulation (83).

The desmopressin test in the postoperative follow-up of patients with Cushing’s disease

Of particular interest is the application of the DT in the postoperative assessment of patients with CD. Despite the suboptimal immediate and long-term cure rates, transsphenoidal surgery (TSS) is the best therapeutic option for patients with CD (64). Remission rates following TSS vary between 70 and 90% (64, 84) and is considerably lower for macroadenomas (64). However, even after initially successful TSS, recurrences may occur in up to 66% of initially controlled patients (64), necessitating frequent and lifelong follow-up. In this context, the DT has been evaluated both during the immediate postoperative period as a predictor of long-term outcome and during follow-up as an early marker of recurrence.

The desmopressin test in the immediate postoperative period as a prognostic marker of the long-term outcome

The recognition of early prognostic markers that can stratify patients according to their long-term risk of recurrence is important for the postoperative management and planning of follow-up of patients with CD in remission. Numerous studies aimed to identify postoperative predictors of long-term outcome; undetectable or very low postoperative cortisol levels, time to recovery of the HPA axis (85, 86), normal late-night salivary cortisol (87), normal cortisol suppression by dexamethasone (88) and CRH test (86, 89) have been used in this context. Although the most widely applied criterion is a low early postoperative cortisol level, a considerable number of patients with low early postoperative cortisol levels recur (90) and a small albeit significant number of patients with higher postoperative cortisol levels achieve late remission (91). Thus, none of the currently widely used
markers is accurate enough in the prediction of long-term remission (64).

The use of the DT postoperatively, at variance with all the other tests that assess the functional activity of the HPA axis, exploits its unique ability to detect the presence of residual neoplastic corticotrophs, and hence, an increased risk for relapse. The existing studies that assessed the usefulness of the DT in this regard are presented in Table 3. In these studies (92, 93, 94, 95, 96, 97, 98, 99), the reported negative predictive value of the test ranged from 76 to 100%, being above 90% in the vast majority of the studies, whereas the sensitivity and specificity to detect recurrence vary greatly from 20 to 100% and from 57 to 100%, respectively. There is considerable disparity, however, among the studies with regards to the definition of response, the applied criteria for remission and recurrence and, the postoperative timing that the DT was performed. Moreover, a few studies do not report on the preoperative response to desmopressin (92, 94, 97), and it is likely that inclusion of desmopressin non-responders compromised the predicting ability of the test. Caution is also needed on the applied criteria of what constitutes a positive desmopressin response. It should be noted that, during the immediate postoperative period, cortisol levels are usually low in those who were successfully operated and even small variations, within the assay CV, may result in significant percentage increases providing erroneously ‘positive’ results. Thus, instead of percentage changes, criteria that rely on absolute values or increments are more robust. In this line, it is of note that in four independent studies (93, 97, 98, 99), assessment of absolute cortisol increments concluded similar cut-off values (7–7.4 μg/dL) to predict the long-term outcome. Combining the data reported in these studies, comprising a total of 116 patients, the negative prognostic value of the DT is 92% with a specificity of 95%, a positive predictive value of 77% and a sensitivity of 68% to predict long-term recurrence.

We recently reported that an increase in serum cortisol of ≥7.4 μg/dL from baseline, following desmopressin administration, had a hazard ratio for recurrence of 24.7 (95% confidence interval, 10.6–448.5) at 60 months (99). Notably, in this series, the predictive ability of the DT was superior from the more commonly used criterion of low or undetectable cortisol level. An additional advantage of the DT was that the loss of the positive preoperative desmopressin responsiveness during the early postoperative period led in the correct assignment of the small subset of patients who do not display early hypocortisolism but undergo delayed remission. In fact, in these patients if the DT becomes negative, a watchful waiting approach may save them from unnecessary repeat surgery.

Altogether, the existing data support a complementary role of the DT performed in the immediate postoperative period, in the assessment of long-term outcome and postoperative follow-up planning of patients with CD. A loss of the paradoxical response indicates a favorable outcome while its persistence necessitates close surveillance for a timely detection of recurrence. The accurate appreciation of the positive predictive value of the DT, however, may be hampered by the fact that the overall number of recurrences in the published studies is small, and they may occur many years after successful surgery. Therefore, larger prospective studies with longer follow-up periods are needed to conclude on this point.

The desmopressin test during follow-up as an early marker of recurrence

In addition to the difficulties in diagnosing CD, at least in some cases, documentation of recurrence at an early stage is also challenging. Several patients with CD when relapsing following TSS remain in a rather low disease activity with marginal biochemical test abnormalities. Early recognition of recurrence before the reappearance of the full spectrum of clinical manifestations or the development of clearly pathological biochemical tests allows timely intervention and prevents patient exposure to the detrimental effects of severe hypercortisolism.

Colombo et al. (93) evaluated 19 patients with CD in post-surgical remission with repeated DTs during follow-up. They did not observe changes in the responsiveness to desmopressin over time; 14 patients considered as ‘cured’ had negative postoperative DT that remained negative and none relapsed, whereas 5 patients with ‘normalization’ of cortisol levels had positive DT at the first postoperative month and remained positive throughout, 2 of them recurred. The length of follow-up, however, was rather short (up to 36 months). Ambrosi et al. (100, 101) described three patients in whom the DT became negative after ‘cure’ but reappeared during follow-up and, importantly, this preceded clinical and hormonal documentation of recurrence by 4–39 months. Bou Khalil et al. (102) also reported that 17 of 20 patients with recurrent CD ultimately had a positive response to vasopressin analogue stimulation (including desmopressin in 17 patients) and that this preceded the increase in midnight cortisol (either serum or salivary) or UFC in 71% of the patients. Recently, Ambrogio et al.
### Table 3  The postoperative desmopressin test in predicting long-term recurrence of CD.

<table>
<thead>
<tr>
<th>Study</th>
<th>No of patients with initial remission tested</th>
<th>No of patients with recurrence tested</th>
<th>Time of follow-up</th>
<th>Positive preoperative DT</th>
<th>Timing of postoperative DT</th>
<th>Criterion for postoperative DT</th>
<th>PPV</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(93)</td>
<td>19</td>
<td>2</td>
<td>1-36 months</td>
<td>19</td>
<td>1, 6, 12, 18, 24 and 36 months</td>
<td>ΔCort &gt; 7.3 μg/dL</td>
<td>100</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td>(96)</td>
<td>64</td>
<td>3</td>
<td>2-54 months</td>
<td>64</td>
<td>5-6 days</td>
<td>ΔCort &gt; 20%</td>
<td>92</td>
<td>16</td>
<td>56</td>
</tr>
<tr>
<td>(95)</td>
<td>164</td>
<td>18</td>
<td>6-148 months</td>
<td>164</td>
<td>4-6 days</td>
<td>ΔCort &gt; 30%</td>
<td>90</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>(98)</td>
<td>150</td>
<td>17</td>
<td>24-141 months</td>
<td>NR</td>
<td>3-6 months</td>
<td>%ΔCORT &gt; 30%</td>
<td>100</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>(97)</td>
<td>41</td>
<td>11</td>
<td>20-161 months</td>
<td>41</td>
<td>15-30 days</td>
<td>%ΔCORT &gt; 20%</td>
<td>90</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>(92)</td>
<td>39</td>
<td>15</td>
<td>24-123 months</td>
<td>NR</td>
<td>6 months</td>
<td>ΔCORT &gt; 30%</td>
<td>90</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>(94)</td>
<td>38</td>
<td>10</td>
<td>18-180 months</td>
<td>NR</td>
<td>3 months</td>
<td>ΔCORT &gt; 30%</td>
<td>90</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>(98)</td>
<td>39</td>
<td>7</td>
<td>12-159 months</td>
<td>39</td>
<td>6 months</td>
<td>Peak ΔCORT &gt; 20%</td>
<td>92</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>(103)</td>
<td>43</td>
<td>10</td>
<td>2-23 years</td>
<td>39</td>
<td>3-6 months</td>
<td>%ΔCORT &gt; 14%</td>
<td>90</td>
<td>100</td>
<td>80</td>
</tr>
</tbody>
</table>

CD, Cushing’s disease; DT, desmopressin test; NA, no available data; NR, not reported; %ΔCort, ((peak cortisol-basal cortisol)/basal cortisol) × 100%; %ΔACTH, ((peak ACTH-basal ACTH)/basal ACTH) ×100%; ΔACTH, peak ACTH-basal ACTH.

### Coupled dexamethasone-desmopressin test (CDDT)

Performing the DT after dexamethasone suppression is a modification that aims to improve the specificity of the test. In fact, time of the testing of cortisol after suppression by dexamethasone should be chosen carefully. In patients with successfully treated CD in the early postoperative period, the HPA axis is already suppressed, and any response that can be observed due to stress, cells and any response that can be observed due to stress, indicates persistence of pathological corticotrophs that may progress over time and lead to clinical reappearance of CD.

Overall, the existing data, although limited, indicate that a positive response might reappear during long-term follow-up earlier than other markers of recurrence, supporting a role of repeated DT as part of the longitudinal evaluation of successfully operated patients with CD. We may progress over time and lead to clinical reappearance of CD.

In patients with successfully treated CD in the early postoperative period, the HPA axis is already suppressed, and any response that can be observed due to stress, cells and any response that can be observed due to stress, indicates persistence of pathological corticotrophs that may progress over time and lead to clinical reappearance of CD.
functionality is gradually restored. In this context, CDDT was explored as a tool to detect recurrence earlier than the conventional hormonal tests. Castinetti et al. (105) reported that an increase of more than 50% in cortisol and ACTH levels after the CDDT had 100% sensitivity and 89% specificity to predict recurrence in 38 CD patients treated by TSS. In this study, the loss of circadian rhythm (no clear definition is given) followed the positivity of CDDT in most patients. A subsequent study by the same group (94) reported similar performance of the DT and CDDT in the early postoperative period; during follow-up, however, the CDDT was more accurate in predicting the lack of recurrence (100% NPV), albeit with a worse positive predictive value, when performed in the first 3 years after surgery. Most importantly, as with DT, the positivity of CDDT was observed earlier than other markers of recurrence.

Conclusions

There is no doubt that the diagnosis and the assessment of therapeutic outcome of CS represent a continuing challenge. The diagnostic performance of most currently available diagnostic tests is compromised by imprecisions due to a high rate of indeterminate results. This is largely due to the fact that tests like the dexamethasone suppression, midnight serum or salivary cortisol and urinary free cortisol excretion are based on the functional status of the HPA axis. Therefore, when applying these tests to detect neoplasia-related cortisol excess, it is not surprising to obtain results overlapping with those found in cases of functional activation of the HPA axis. In contrast, the positive responses to vasopressin do not relate to a functional activation of the HPA axis, but they are only associated with changes in vasopressin receptor expression that develop in ACTH-producing cells undergoing neoplastic transformation. That being so, DT is not useful for the differentiation between CD and EAS, where BIPSS remains the gold standard. Its unique receptor expression that develop in ACTH-producing cells makes the DT a useful additional tool in the diagnosis and postoperative follow-up of patients with CD. In fact, due to its high specificity the DT provides further insight favoring the diagnosis of CD when other tests are inconclusive. Furthermore, there is accumulating evidence that it may aid the postoperative assessment of these patients. It is therefore suggested that the DT should be more widely incorporated as an adjunctive tool in the preoperative and postoperative investigation of patients with CS; additional large prospective studies will establish its exact role.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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