Indication and Technical Aspects of Adrenal Blood Sampling

Indikationen und Technik der selektiven Nebennierenvenenblutentnahme

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● abdomen
● adrenal gland
● angiography
● endocrine disorders

Abstract
Adrenal disorders are relative frequent with regard to the incidence of adrenal tumors and the high portion in causes of secondary hypertension. Morphological changes in the adrenal glands can lead to very different functional disorders that may be clinically overt or hard to diagnose. On the other hand, they can already be functionally relevant when structural changes are too small to be picked up by imaging. Adrenal venous sampling serves to determine the source of hormone excess through the analysis of adrenal blood. In this manuscript, we call attention to the clinical backgrounds, critical points in practice, technical aspects and developments in the field of adrenal venous sampling. The consideration of these important points in the clinical setting may make adrenal vein sampling studies successful and help to select patients that qualify for adrenalectomy.

Zusammenfassung

Introduction
Since evidence of an adrenal tumor does not automatically identify the source of hormone excess, and tomography cannot always pick up small but relevant endocrine adenomas (< 1 cm), selective adrenal venous sampling (AVS) is the gold standard for the identification of patients with primary aldosteronism who can benefit from adrenalectomy. Knowledge of the anatomy and normal variants of the adrenal veins facilitates adrenal venous sampling.

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and clinical aspects the observance of which in clinical practice has contributed to the success of this investigation.

Disorders that can be better characterized using selective adrenal vein catheterization

1. Primary aldosteronism (Conn’s syndrome, PA)

Primary aldosteronism (PA), with a prevalence of 5–10% is the most frequent secondary form of hypertension [4–6]. PA is particularly frequent (10–20%) in patients with difficult to manage hypertension, making up approximately 10–30% of patients with high blood pressure [7]. The possibility of Conn’s syndrome should be excluded for those patients for whom renal nerve ablation is considered. Primary aldosteronism does not exhibit a homogeneous disease pattern. In addition to various familiar forms, recently several somatic mutations have been discovered that contribute to the development of this disorder [8–11]. Further, the differentiation of unilateral from bilateral disease is of particular clinical significance due to the related therapeutic options. It should be presumed a diagnosis of bilateral hyperplasia (BAH) is provided in approximately 50% of patients, whereas in the remaining cases an aldosterone-producing adenoma (APA) and asymmetrical aldosterone excess is present. Rarer entities include adrenal cortex carcinoma, which occurs in <1% of cases [12, 13].

Patients with Conn’s syndrome have a significantly increased risk of cardiac and vascular diseases, even when compared with patients with similarly poor blood pressure values, but without hyperaldosteronism [14, 15]. Therefore is it worthwhile for those affected patients to follow the route of multi-step diagnosis consisting of screening examinations, functional tests, imaging and successful adrenal venous catheterization [16–18]. This is reflected in an elevated aldosterone to renin ratio, which is also appropriate as a screening examination as long as no anti-hypertensive medications have been taken, as these intervene in the renin-angiotensin system (Fig. 1); such an examination should take into account cyclical phases and exclude as well other bases for a false positive laboratory result such as hyperkalemia, infection, stress, etc.

If the renin level is low while aldosterone is inadequately high (e.g. > 20 µg/l:µg/l), a confirmation test must be performed due to the limited specificity of the screening examinations [16]. The sodium chloride load test and fludrocortisone suppression test are primarily suitable for this purpose. However, since the tests demonstrate sensitivity and specificity between 80% and 90% [16], other aspects such as a typical history, concomitant medication during the test, or hypokalemia (in free-flowing blood) must be taken into account. Since the fludrocortisone suppression test appears to provide better sensitivity and produces fewer false negative results, in cases of doubt results of this test should be given more weight than those of others [19, 20]. If the diagnosis has confirmed Conn’s syndrome, the next step is investigate which adrenal gland is the source of aldosterone excess [16]. Since the findings of functional diagnostics and imaging too frequently differ, a selective AVS should be performed; this procedure was first described in 1967 [21] and currently represents the gold standard for differentiating various abnormality subtypes [22–24].

As a result of a systematic analysis, approx. 37% of all 950 reported patients would have undergone incorrect treatment if only CT or MRI had been used for localization diagnostics [25]. Of these, about 15% would have undergone surgery as a consequence, even though bilateral disease was present; 3.9% would have had surgery on the wrong side, whereas an adrenalectomy would have been withheld for 19%, despite lateralized aldosterone excess. There are various reasons for the limited value of MRI or CT tomography. Hormonally-inactive adrenal masses occur more frequently with increasing age; this has practical importance above the age of 40. On the other hand, adenomas forming aldosterone are sometimes very small (<5 mm) and can escape detection [26, 27]. In a collective study,
Stowasser et al. found that more than 50% of aldosterone-producing adenomas were less than 1 cm in size [22]. A CT of the adrenal glands is nevertheless useful, as it can rule out adrenal cortex carcinoma while displaying the anatomy of the adrenal veins. Adrenal venous sampling plays a decisive role in the differentiation of the two major entities of Conn’s syndrome, aldosterone-producing adenoma (APA) and bilateral hyperplasia (BAH).

2. Bilateral adrenal tumors, adrenal Cushing’s syndrome and other indications

While aldosterone-producing tumors are sometimes too small to be detected by radiological diagnostics, and there appear to be two seemingly normal, although somewhat hyperplastic adrenal glands in the image, adrenal cortex lesions that are the source of Cushing’s syndrome are clearly visible, with few exceptions. However, time and again, two adrenal tumors occur. In addition to cortisol-producing adenoma (CPA), manifestations can also include simultaneously present non-hormonally active tumors, pheochromocytomas or ACTH-independent macronodular adrenal hyperplasia (AIMAH, Fig. 2). Whereas in the past, operations were first performed on the side with the visibly larger tumor [28, 29], improved and expanded analytical methods have enabled the use of an adrenal vein catheter to identify the more hormonally productive side as well as hormonal characterization of adrenal cortex tumors [30–32].

Cushing’s syndrome likewise exhibits a smooth transition from minor symptoms to the well-known clearly visible syndrome, so that multi-stage diagnostics can be utilized employing various medications and synthetic hormones, while taking into account diurnal fluctuations [18]. Further, in cases of Cushing’s syndrome (especially in its milder form), it should always be critically considered whether the tested hormone values actually reflect the clinical condition of the patients and their symptoms and whether an operation and interventional investigation will aid in diagnosis. Existing guidelines and recommendations provide practical assistance in this regard [33, 34].

An additional option for utilizing AVS is the identification of the source of hyperandrogenemia. Thus in the case of bilateral adrenal disorders, an attempt can be made to identify the dominant side. If the ovarian veins are likewise cannulated, then it can investigated whether a small androgen-producing Leydig cell tumor is present, or whether the source is of adrenal origin [35]. For the sake of completeness, it should be mentioned that AVS has also been used to investigate pheochromocytomas. Due to potential hazards, this procedure is being increasingly abandoned.

Adrenal venous sampling technique

A study by Mulatero et al. showed that aldosterone-producing adenomas are more frequently found in centers where AVS is regularly performed [5]. At > 95%, the sensitivity and specificity of AVS is very high if selective blood sampling succeeds. Even in specialized centers, the technical success rate is only 70–90%; at the lower range the quality is comparable to conventional tomography [36]. Due to the diagnostic effort and life-long therapeutic consequences, AVS must therefore be successful, and is a highly important element in the investigation of patients with Conn’s syndrome. Beyond that, AVS is also suitable for better characterization of other adrenal disorders. The prerequisites for a high success rate are the experience of the radiologist as well as good knowledge of anatomy and related standard variations (Fig. 3). Selective adrenal blood sampling is frequently viewed as difficult and time consuming due to three different reasons. The first reason is due to the small size of the right adrenal vein; the second is a result of lack of knowledge of angiographic images of adrenal veins and the resulting difficulty in distinguishing other vessels which may lead into the posterior wall of the inferior vena cava (IVC). The third reason is related to the length of the segment of the inferior vena cava which, due to the high variance of adrenal vein anatomy, can extend from the 10th to the 12th intercostal space. To ensure the proper position of the catheter, careful display of the vein should utilize a contrast agent administered via the catheter (Fig. 4). Display of the proper catheter position using a C-arm CT is a current additional option. Perfect positioning

![Unenhanced CT of the upper abdomen phase of a 44-year-old patient with ACTH-independent macronodular adrenal hyperplasia (AIMAH).](image)

![Significant elongation of the exemplified right adrenal veins during AVS.](image)
should permit selective sampling of the entire adrenal gland; this is difficult, however [37]. It is particularly important to distinguish the right adrenal vein leading directly into the inferior vena cava from small accessory hepatic veins (Fig. 5).

The earlier method involving blood sampling from the adrenal glands, the IVC above and below the adrenal veins and additional periphery can be abandoned in favor of a simple peripheral control sample obtained via the bleedback prevention valve inserted in the inguinal vein.

### Adrenal gland vein anatomy

Venous drainage of the adrenal glands occurs bilaterally via a central vein [38]. Cadaver studies have indicated an average length of 1–15 mm for the right adrenal vein and 10–40 mm to the confluence with the phrenic vein for the left adrenal vein [39–41]. The central vein of the right adrenal gland may be duplicated or even tripled, and one of these veins can empty into the inferior phrenic vein or the renal vein, but one vein always leads into the inferior vena cava. Central adrenal gland veins with intrahepatic connections are rare; in the literature the incidence of these variants differ, between <1–10%. Presumably this phenomenon is seldom encountered [39–42], and there should be no confusion regarding the hepatic juncture of peripheral adrenal veins (see below).

The left central adrenal gland vein generally joins the inferior phrenic vein forming a common trunk of variable length and enters the left renal vein from above [38–40]. Only rarely do the left adrenal gland vein and the inferior phrenic vein drain separately into the renal vein.

Both adrenal glands have capsule veins emanating from the surface which are joined with intercostal or phrenic veins, and – more rarely – with hepatic veins (Fig. 6). On the left side, there may be connections to the (hemi) azygos veins, to the renal vein or the inferior vena cava (Fig. 7). Angiographic representation of these superficial veins supports the detection of a proper catheter position.

The anatomical variations of the left renal vein and inferior vena cava are significant for probing the left adrenal vein. These can be identified easily using CT in the venous perfusion phase. This is a relevant argument for performing a CT of the adrenal glands and renal anatomy prior to selective adrenal vein sampling to avoid unexpected difficulties during catheterization and to allow the left adrenal vein to be probed without loss of time.

Using a thin-slice CT (SD 2 mm), identification of the right adrenal vein is possible in 50% of patients. The CT is used to assess the position of the adrenal vein, in the DSA, the outlet for the vein should be considered to be 1–1.5 cm upward from the position indicated in the CT image. If the...
right adrenal vein cannot be differentiated in the CT, the center of the adrenal gland should be used to estimate the position of the vein [43].

Blood vessel imaging of the right adrenal gland shows, in addition to variances, recurrent vascular patterns which are described below.

- The vessel image can be represented glandularly with a central main vessel and several branches, which typically emanate at a < 90° angle from the trunk [39, 40, 42] (Fig. 8a).
- The adrenal veins can be arrayed in classic delta form, superficial veins can also be displayed (Fig. 8b).
- An angiographic image can display triangularly arranged veins; the branches of the adrenal gland are rather densely delineated in the upper angle.
- Contrast imaging of the adrenal veins can be very subtle, so that a glandular constellation of blood vessels is not recognizable; sometimes only a main trunk and individual superficial veins can be displayed (Fig. 8c).
- The vein formation can be spider-like with a central vein and lateral branches as well as superficial veins reminiscent of a spider (Fig. 8d).

Differentiation from small accessory hepatic veins can be made by somewhat protracted application of the contrast agent; after a few seconds, liver tissue shows homogeneous contrast (Fig. 5), whereas the fine branches of the adrenal gland remain, but without planar contrast. An adenoma can also be differentiated in an image during selective adrenal vein sampling. In the case of apparent hyperplasia of the adrenal glands, the angiographic image will display a corresponding vessel pattern up to elongated veins (Fig. 2).

**Catheter material**

To perform selective adrenal vein sampling, a 6F bleedback prevention valve should be inserted inguinally into the femoral vein. The catheter, either 5F or 4F, is inserted into the inferior vena cava up to the height determined in the CT, and rotated dorsally. Probing is performed by arching up to 45° to the right.

A C1 or SIM 1-configured catheter is recommended for probing the right adrenal gland (Fig. 9). The VAN catheter (Boston Scientific) uses SIM 1 configuration with additional...
lateral perforations near the catheter tip so that blood can easily be sampled when the catheter tip lies against a vein wall. Lateral perforations can also be created manually on the catheter tip using a trocar. The path of the right adrenal vein runs caudally. When a SIM 1 or VAN catheter is used, it is rotated once along the longitudinal axis once it has hooked into the vein ostium so that the catheter tip points caudally rather than dorsally (Fig. 9b), thus lying securely in the adrenal vein. For the sake of completeness, the Mikaëlsson catheter should be mentioned; this likewise does not possess additional lateral perforations.

In principle the left adrenal vein can be probed using various catheters; in addition to C1 or C2 configurations, probing is also possible using a vertebral catheter [43, 44]. In our opinion, a SIM 2 Glidex catheter provides simpler and faster probing; depending on its configuration in the inferior vena cava or right chamber, if the catheter is withdrawn with the tip pointing left, it automatically lies in the left renal vein, and then upon additional careful withdrawal will rest in the left adrenal vein or the common end section (Fig. 10). Due to usually large vein caliber, a catheter with a simple end perforation suffices in this case; additional side perforations are not required for the left adrenal vein.

When imaging the veins to confirm the proper catheter position, the contrast medium should be carefully injected and discontinued as soon as a typical blood vessel pattern

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**Fig. 7** a Shows a juncture of left-side superficial adrenal veins with the renal vein. b Shows a juncture with the inferior vena cava below the confluence of the renal vein.

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**Fig. 8** a Gland-like aspect of the right-side adrenal veins. b The vessels of the right adrenal gland exhibit a typical delta form; in this case superficial veins are not visible. c The central adrenal veins are not shown, only a few superficial veins are opaque; nevertheless the correct position of the catheter must be determined using these veins. d The superficial veins exhibit a spider-like configuration. The central veins are only discreetly opaque.
is discerned. A maximum of 3 ml contrast medium should be necessary. Application of too great pressure may perforate adrenal vein branches. Sometimes it is possible to see vessel structure displaced by an existing adenoma (Fig. 11), but this is not the purpose of using a contrast agent which should be used solely for documenting the proper catheter position. Care should be taken to ensure that the catheter does not lie too deeply in the adrenal vein so that only blood is sampled from a branch of the adrenal gland. This could lead to a false negative result (Fig. 12). This pitfall again emphasizes the necessity of administering a contrast medium, as it is the only means to recognize such an error and thus avoid or correct it.

### Blood sample evaluation

First the ratio of cortisol concentration in the adrenal vein (AV) and peripheral vein (PV), e.g. blood from the bleeding prevention valve, is determined [16]; this is designated the selectivity index \((SI = \text{Cortisol}_{AV} / \text{Cortisol}_{PV})\). If the SI is > 2.0, then this ratio can be used for calculation since the portion of adrenal vein blood sampled is sufficiently high. If the SI < 2.0, then the blood may not have drained from an adrenal gland, or is blood from an adrenal vein that is contaminated with too much blood from other veins.

In order to prove lateralization, the aldosterone–cortisol ratios for both adrenal veins (AV) are determined in a second step and then compared (aldosterone\(_{AV \text{ Side } 1} \div \text{cortisol}_{AV \text{ Side } 1} / \text{aldosterone}_{AV \text{ Side } 2} \div \text{cortisol}_{AV \text{ Side } 2})\). If the ratio is > 3, then according to the recommendations of the Conn's Registry, there is a probability of APA (Table 1). If the lateralization index (LI) is > 2, then there is unilateral disease [45]. If one side cannot be sufficiently cannulated selectively, calculation of the contralateral suppression index (CSI) can sometimes indirectly verify asymmetrical aldosterone secretion [45]. An aldosterone-cortisol ratio in the sample taken from the adrenal vein less than that in the sample of peripheral blood can be interpreted to be an indication of APA on the contralateral side, and in the appropriate clinical context (such as a typical finding in the imaging study) can be assessed as unilateral disease.

### Adrenal venous sampling variations

One variant uses the administration of Synacthen (synthetic signal peptide of ACTH in a 50 µg/h infusion). An advantage of ACTH infusion started prior to the actual catheterization is constant adrenal stimulation which levels circadian variations, thus allowing the examination to be performed later that day or if there are longer interruptions (if, for example several tests must be performed) [46]. There is a further advantage for patients with known contrast agent allergies,
since dexamethasone can be administered prior to the ACTH infusion. Due to cross-reactivity in immunoassays, administration of prednisolone in such cases is a mistake, however.

Since the ACTH infusion increases the concentration gradient for cortisol and aldosterone from central to peripheral, other threshold values apply to the selectivity and lateralization indices. In this case, an SI > 3 should be presumed, above which an LI > 4 would be sufficient indication of unilateral disease [45]. We see problems related to this, since the availability of Synacthen is currently quite limited due to the sale of production rights [47]. Further, the distinction between unilateral and bilateral disease can easily be blurred, since normal adrenal tissue responds with raised aldosterone secretion after administration of ACTH [48]. Because of this, we perform AVS without ACTH stimulation at our Center.

Last year a study was published indicating the possibility to investigate the selectivity of AVS with more sensitivity [33]. Based on the assumption of SI > 2.0 for cortisol, we had an approximate 80% success rate for bilateral AVS, while the determination of free metanephrine in the plasma indicated that the success rate certainly lay > 90%, and there were even assessable studies for which the SI for cortisol was < 2.0. The SI for plasma metanephrine during mass spectrometry measurement should be assumed to be above 12. These threshold values for metanephrine certainly should not apply to immunoassays. Whether increased sensitivity can be utilized to detect lateralized aldosterone excess more sensitively is currently under investigation.

There is an additional possibility of measuring other steroid hormones that might be more specific to aldosteronomas, since adrenal tumors frequently acquire steroid biosynthesis disorders. This ratios could derived from 18-hydroxy-corticosterone and aldosterone or cortisol [49], or 18-oxo-cortisol to cortisol [50] in order to identify an APA with more certainty.

Currently, within the context of the Conn’s Registry (www.conn-register.de) and in conjunction with other European centers, research is being conducted to determine whether steroid profiles consisting of > 15 steroid hormones and intermediary hormones are indicative of an aldosteronoma. In addition, there are studies to determine whether different steroid patterns are caused by various mutations resulting in the development of APAs.

**Key statements regarding successful adrenal vein catheterization and current developments**

- The diagnostic process undergone by the patient prior to an AVS costs time (multi-stage diagnostics), poses risks (change of medication, functional diagnostics) and is related to a period of disease that frequently long predates diagnosis, and in some cases is fraught with complications. For the patient, the clinical effort is worthwhile particularly if it results in the definite diagnosis of unilateral disease, as this supports the hope for a cure, clinical improvement, or at the least a reduction of the number and dosage of antihypertensive medication.

### Table 1

Example 1 shows hormone concentrations in the blood obtained by selective adrenal venous sampling together with an example for calculation of the lateralization index, which shows dominant aldosterone secretion from the left adrenal vein. Example 2 shows hormone concentrations obtained by adrenal venous sampling which was formally successful on the left side only. However, calculation of the contralateral suppression index (< 1 on the left side) indirectly locates excess aldosterone secretion to the right side.

#### example 1

<table>
<thead>
<tr>
<th></th>
<th>right</th>
<th>left</th>
</tr>
</thead>
<tbody>
<tr>
<td>A in ng/l</td>
<td>1401</td>
<td>439</td>
</tr>
<tr>
<td>F in µg/dl</td>
<td>236.8</td>
<td>22.9</td>
</tr>
<tr>
<td>SI</td>
<td>10.3</td>
<td>17.5</td>
</tr>
<tr>
<td>A/F</td>
<td>5.9</td>
<td>–</td>
</tr>
<tr>
<td>LI</td>
<td>–</td>
<td>89.0</td>
</tr>
</tbody>
</table>

- dominant left area, because 89.0 / 5.9 > 3

#### example 2

<table>
<thead>
<tr>
<th></th>
<th>right</th>
<th>left</th>
</tr>
</thead>
<tbody>
<tr>
<td>A in ng/l</td>
<td>145</td>
<td>198</td>
</tr>
<tr>
<td>F in µg/dl</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>SI</td>
<td>0.8</td>
<td>–</td>
</tr>
<tr>
<td>A/F</td>
<td>–</td>
<td>4.2</td>
</tr>
<tr>
<td>KSI</td>
<td>–</td>
<td>0.11</td>
</tr>
</tbody>
</table>

- the contralateral suppression index in the left side is assured < 1

A – aldosterone, F – cortisol, KSI – contralateral selectivity index, LI – lateralization index, NNV – adrenal vein, SI – selectivity index. Explanations on the calculation models are found with the text of the manuscript.

![Fig. 11 a Axial CT in the venous phase exhibiting a small right-side adenoma. b Sometimes an adenoma can be distinguished in the DSA (arrows).](image)
First, the patient should be well informed at the onset of the examination, so that he can deal with potential failure while maintaining motivation to undergo further testing.

Since the hormones under investigation show strong diurnal fluctuations, the examination should be performed early in the morning after the patient has been reclining for an extended period, as this will level out differences and decrease the specificity of the obtained data [45, 51].

An attempt should be made to obtain sufficient blood from the adrenal veins (approx. 10 ml), since this may indicate an extremely high level of hormones under normal conditions, and may have to be thinned out multiple times. Attention should also be paid to pre-analysis, since a single questionable hormone value, be it due to improper sample storage, poor assay for these hormone values or ambiguous labeling of a blood sample, can make an entire study worthless.

It should likewise be taken into account that larger APAs can also secrete relevant quantities of cortisol such that lateralization due to cortisol co-secretion can no longer be identified computationally [13]. Further, the selectivity index on the contralateral side will be erroneously low. Consequently a thorough history should be taken of patients with Conn’s syndrome together with a physical examination; in cases of doubt a dexamethasone inhibition test should be performed.

Selective sampling from the right adrenal vein is best performed with a VAN catheter; time can be saved by probing the left adrenal vein using a SIM 2 catheter with a Glidex tip which is sufficiently effective.

We recommend careful application of a contrast agent to aid in displaying the position of the catheter; in this way the anatomy and its variants (see above) as well as pitfalls can be detected, and the selective AVS will enjoy a high success rate. A rapid cortisol assay will also provide verification of the result.

The rapid cortisol assay is the direct determination of cortisol in additionally obtained blood samples (plasma) from the initially cannulated right adrenal vein and from the bleedback prevention valve; it takes 20 to 45 minutes [52, 53]. During this time the less problematic left side can be probed. If the cortisol level in the blood from the adrenal vein is double that of the peripheral blood sample (SI > 2.0), then a successful assay can be presumed. If it is lower, then it is definitely advisable to take a second or third sample from the right adrenal vein (see above), since the “hit rate” can be thus increased [52, 53].

For the sake of completeness we would like to mention that a working group recently demonstrated that an 11C-metomidate-based PET-CT examination is sufficiently sensitive to display even small aldosteronomas and is largely consistent the results of an AVS [54, 55]. Initial efforts to establish similar diagnostic methods in Germany have already been undertaken, but the desired resulting sensitivity will not be available in the foreseeable future [56].

In summary, selective adrenal venous sampling (AVS) currently remains the gold standard for the identification of patients with primary hyperaldosteronism who can profit from adrenalectomy.
Review

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