

CLINICAL STUDY

Adrenal venous sampling in primary aldosteronism: a low dilution of adrenal venous blood is crucial for a correct interpretation of the results

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Abstract

Objective: In primary aldosteronism, adrenal venous sampling (AVS) is essential for subtype differentiation as it evaluates aldosterone secretion from both adrenals. Selectivity of adrenal sampling is assessed by the ratio of cortisol concentrations in adrenal venous blood and inferior vena cava blood ($C_{\text{adrenal}}/C_{\text{ivc}}$). Since the criteria for selective adrenal sampling differ among the reported literature, we performed a study to evaluate the influence of different selectivity criteria on AVS results.

Design and methods: Reports of AVS were screened retrospectively. All AVS were performed with cosyntrophin infusion. Reports containing samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 10$ taken from both adrenals and at least one other adrenal sample characterised by $C_{\text{adrenal}}/C_{\text{ivc}} \geq 1.1$ were enrolled. For each individual, we chose reference samples that were defined by the highest $C_{\text{adrenal}}/C_{\text{ivc}}$ achieved from each adrenal. The significance of the remaining samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 1.1$ was analysed in regard to their respective reference samples. We assessed the impact of analysed samples on identification of lateralisation of aldosterone secretion that is crucial for decisions concerning adrenalectomy.

Results: AVS reports of 87 patients were enrolled. A total of 225 adrenal samples were analysed and divided into five groups according to $C_{\text{adrenal}}/C_{\text{ivc}}$: 1.1–1.99, 2–2.99, 3–4.99, 5–9.99 and ≥ 10 . By comparing reference with analysed samples, a concordant assessment with respect to lateralisation of aldosterone secretion was observed in 39, 52, 72, 85 and 94% of the respective groups of analysed samples.

Conclusion: AVS provides consistent information when adrenal samples with high cortisol concentrations are used.

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Introduction

Primary aldosteronism represents a frequent and potentially curable form of secondary arterial hypertension. Provided there is unilateral aldosterone overproduction, adrenalectomy leads to improvement or even normalisation of elevated blood pressure in the majority of patients (1, 2). The only diagnostic method that can, in principle, differentiate between unilateral and bilateral aldosterone hypersecretion is adrenal venous sampling (AVS), with samples taken from both adrenal veins (3, 4). The success rate of the AVS procedure depends on an accurate cannulation of both adrenal veins. Since a high cortisol concentration is an explicit determinant of the adrenal venous blood, the adequacy of AVS is usually evaluated by the ratio of the cortisol concentrations in the adrenal vein and in the inferior vena cava ($C_{\text{adrenal}}/C_{\text{ivc}}$). High $C_{\text{adrenal}}/C_{\text{ivc}}$ is a result of less dilute blood from the adrenal veins, which is a more representative sample of the adrenal venous blood.

As the production of cortisol by both adrenals is considered symmetrical, cortisol-corrected aldosterone concentrations (ACs) are used to compare aldosterone production from both adrenals. The aldosterone-to-cortisol concentration ratio (AC) is intended to eliminate the variable dilution effect when adrenal samples are taken. The lateralisation of aldosterone secretion is measured by the ratio of the ACs from both the adrenals (ACR), and is defined as $AC_{\text{dominant adrenal}}/AC_{\text{non-dominant adrenal}}$.

The arbitrary criteria for interpreting AVS data differ greatly among the published literature (5–10). The current guidelines for adrenal sampling define the adrenal/peripheral vein cortisol ratio as typically $>10:1$ with the continuous infusion of cosyntrophin, and more than $3:1$ without cosyntrophin (11). The published criteria for appropriate adrenal vein sampling range from $C_{\text{adrenal}}/C_{\text{ivc}} \geq 2-5$ with cosyntrophin (12, 13) and from $C_{\text{adrenal}}/C_{\text{ivc}} \geq 1.1-3$ without cosyntrophin (14, 15).

Accepting a lower $C_{\text{adrenal}}/C_{\text{ivc}}$ cut-off increases the success rate of the AVS procedure; however, this approach carries a potential risk of misleading AVS results due to excessive dilution of the adrenal venous blood. On the other hand, a higher $C_{\text{adrenal}}/C_{\text{ivc}}$ cut-off increases the number of patients with unsuccessful AVS. It is evident that, among patients with unsuccessful AVS, there must be a significant portion of those for whom adrenalectomy is inappropriately denied.

We evaluated the accuracy of various $C_{\text{adrenal}}/C_{\text{ivc}}$ cut-offs that are used to identify representative adrenal samples as an indication for adrenal surgery.

Subjects and methods

The AVS protocols of patients who underwent successful adrenal catheterisation were screened retrospectively. The included reports contained samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 10$ taken from both adrenals, and at least one other sample with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 1.1$.

Indication of AVS resulted from a high clinical suspicion of primary aldosteronism, which was based on a previous non-invasive diagnostic work-up. Withdrawal of aldosterone antagonists for 2 months was required before the procedures were initiated. The aldosterone-to-active renin concentration ratio (ARR) was used to screen patients for primary aldosteronism. Blood sampling for ARR was always performed in the morning hours (0700–1000 h) with the patients in the upright position for at least 30 min before sample collection. For calculation of ARR, both serum aldosterone and plasma renin concentrations were expressed in ng/l. $\text{ARR} > 20$ was considered to be an abnormal value, which was the cut-off value based upon our observations in 69 healthy volunteers (M Solar, J Ceral and E Malirova, unpublished data). Before sampling for ARR, no specific changes in antihypertensive medication were initially required (except for the withdrawal of any aldosterone antagonists as mentioned above).

However, if the patient presented with an $\text{ARR} > 20$, adrenal adenoma or hypokalaemia, ARR sampling was repeated after 2 weeks of withdrawal from any medication that could interfere with the renin–angiotensin–aldosterone system. Only doxazosin and verapamil were allowed for patients who were considered at risk from uncontrolled hypertension.

Based on persistent elevation of ARR (> 20), the patients were referred for suppression testing.

Verapamil and doxazosin were the only antihypertensive drugs allowed. Blood samples for serum aldosterone were taken in the supine position after a 4 h saline infusion (2000 ml) and a previous period of high salt intake. Aldosterone was considered non-suppressible when it was > 100 pmol/l, which was the cut-off value based on our observations from 32 healthy volunteers (M Solar, J Ceral and E Malirova, unpublished data).

All AVS procedures were performed according to the routine standardised protocol used in our centre. No changes in concomitant medications were required, except for oral anticoagulants and aldosterone antagonists that had to be withdrawn before the procedure. All of the patients were given informed written consent for the AVS procedure according to the following protocol. In order to minimise stress-induced fluctuations in secretion of adrenal hormones, an infusion of cosyntrophin was administered 30 min before and during the procedure at a rate of 160 $\mu\text{g}/\text{h}$. The design of the cosyntrophin dosing scheme was inspired by previous reports (3, 4). Samples were taken step by step from both adrenal veins, one of the hepatic veins, and the inferior vena cava below the renal veins. Hepatic and inferior vena cava samples served as controls, which helped us to obviate the origin of all samples collected.

Because the cannulation of the adrenal veins (especially the right adrenal vein) is technically difficult, multiple samples were taken in order to increase the success rate of the procedure.

Angiographic verification of the catheter placement into the adrenal veins was required both before and immediately after sampling in order to exclude the possibility of catheter displacement during blood sampling. Small amounts of a contrast agent were used to diminish the risk of adrenal injury.

In order to minimise uncertainties when referring the patients for adrenalectomy, we adopted the strict criteria published by Young *et al.* (13). The AVS procedure was considered successful when samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 5$ were obtained from both adrenals. Abnormal lateralisation of aldosterone secretion was then defined by $\text{ACR} > 4$.

A reference sample for each adrenal was chosen from each patient included in our study. The reference samples were defined as the highest $C_{\text{adrenal}}/C_{\text{ivc}}$ achieved from a given adrenal. The remaining adrenal samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 1.1$ were subjected to analysis.

Based on the concentrations of aldosterone and cortisol in the reference samples, $\text{ACs}_{\text{reference}}$ and $\text{ACRs}_{\text{reference}}$ were calculated for each individual. The concentrations of aldosterone and cortisol from the analysed samples were used to calculate the $\text{ACs}_{\text{analysed}}$ and $\text{ACRs}_{\text{analysed}}$. The $\text{ACRs}_{\text{analysed}}$ were calculated from the $\text{ACs}_{\text{analysed}}$ and contralateral $\text{ACs}_{\text{reference}}$.

Analysed samples were divided into five groups according to the $C_{\text{adrenal}}/C_{\text{ivc}}$ levels: 1.1–1.99, 2–2.99, 3–4.99, 5–9.99, and ≥ 10 . In each group of analysed samples, the $\text{ACs}_{\text{analysed}}$ were correlated with the corresponding $\text{AC}_{\text{reference}}$ (i.e. from the same adrenal). The comparison of $\text{ACRs}_{\text{analysed}}$ and respective $\text{ACRs}_{\text{reference}}$ was used to assess the impact of analysed samples on the interpretation of the AVS results.

In order to offer applicable information for centres using different ACR cut-offs (5, 10), we tested the impact of different ACR cut-offs on the agreement between the

analysed and reference samples in the identification of abnormal lateralisation of aldosterone secretion.

Aldosterone and cortisol concentrations were measured from all of the acquired samples. Commercially available RIA kits were used to measure aldosterone (Diagnostic Products Corporation, Los Angeles, CA, USA) and cortisol (Immunotech, Beckman Coulter, Marseille, France). In the long term, we observed the intra-assay coefficients of variation (CV) to be < 7% for the measurements of aldosterone and not more than 5% for the measurements of cortisol. The cross-reactivity of the diagnostic antibodies for other steroids is low.

MedCalc (version 9, Mariakerke, Belgium) software was used for statistical analysis of the acquired data.

Results

Between May 2005 and November 2008, 116 patients underwent AVS in our centre. The procedure was successful in 106 (91.3%) of these patients. Adrenal haemorrhage occurred in one patient. This adverse event resolved without a negative sequel. No other complications were noted.

The AVS protocols of 87 patients who fulfilled the entry criteria were enrolled in the study. The principle characteristics of the study group are summarised in Table 1. Lateralisation of aldosterone secretion, according to the above-mentioned criteria, was found in 25 (29%) of the study patients. A total of 225 adrenal samples were subjected to the analysis. The analysed samples were divided into five groups according to the C/C_{ivc} , as shown in Table 2.

Correlation of analysed and reference samples

Cortisol-corrected ACs from the analysed samples were compared with their respective reference samples. No correlation ($r=0.05$) was noted in the analysed samples with $C_{adrenal}/C_{ivc}1.1-1.99$. Weak correlations were

Table 1 Principal characteristics of the study population.

Total number of patients (number of women)	87 (22)
Age (years)	51.5 (±11.2)
Systolic blood pressure (mmHg) ^a	143 (±20)
Diastolic blood pressure (mmHg) ^a	84 (±15)
Number of antihypertensive drugs ^a	3.9 (±1.4)
Number of patients with abnormal CT or MR adrenal finding ^b	41 (47.1%)
Serum aldosterone (pmol/l) ^c	250 (±160)
Plasma active renin (ng/l) ^c	2.0 (±1.6)

^aDescribes the data obtained before the screening tests were done.
^bDenotes a finding of adenomas, nodularity or marked asymmetry in the size of adrenal glands.
^cAfter suppression performed by 2000 ml saline infusion given during 4 h with a previous high oral salt intake. Values are expressed as the number of patients or mean (±s.d).

Table 2 Characteristics of analysed samples.

	Analysed samples				
	$C_{adrenal}/C_{ivc}1.1-1.99$	$C_{adrenal}/C_{ivc}2-2.99$	$C_{adrenal}/C_{ivc}3-4.99$	$C_{adrenal}/C_{ivc}5-9.99$	$C_{adrenal}/C_{ivc}≥10$
Number of analysed samples (right sided)	44 (42)	25 (23)	25 (25)	20 (10)	111 (58)
$C_{adrenal}/C_{ivc}$ of analysed samples	1.41 (1.10-1.94)	2.60 (2.00-2.98)	3.62 (3.00-4.89)	8.46 (6.19-9.69)	20.00 (10.00-86.84)
$C_{adrenal}/C_{ivc}$ of ipsilateral reference samples	28.32 (13.00-80.70)	24.46 (10.60-80.70)	32.38 (10.23-70.59)	16.45 (11.11-72.50)	25.54 (11.40-94.74)
$C_{adrenal}/C_{ivc}$ of contralateral reference samples	18.01 (10.60-54.33)	20.30 (11.04-20.30)	24.55 (13.93-90.00)	17.44 (10.00-70.00)	23.94 (10.23-94.74)

Analysed samples were divided into five groups according to the $C_{adrenal}/C_{ivc}$. Reference samples were defined by the highest $C_{adrenal}/C_{ivc}$ achieved in a given adrenal. Ipsilateral and contralateral reflect the relation to the analysed samples. Values are expressed as number of samples or median (range). $C_{adrenal}/C_{ivc}$ represents the ratio of cortisol concentrations in the adrenal vein and in the inferior vena cava.

observed in samples with $C_{\text{adrenal}}/C_{\text{ivc}} 2-2.99$ ($r=0.37$) and $C_{\text{adrenal}}/C_{\text{ivc}} 3-4.99$ ($r=0.32$). Much higher correlations were exhibited from the analysed samples with $C_{\text{adrenal}}/C_{\text{ivc}} 5-9.99$ ($r=0.88$) and $C_{\text{adrenal}}/C_{\text{ivc}} \geq 10$ ($r=0.90$).

Comparison of analysed and reference samples – clinical impact

Comparisons of both the analysed and reference samples that were used in the identification of lateralisation of aldosterone secretion (defined as $\text{ACR} > 4$) are shown in Table 3. The analysed samples with $C_{\text{adrenal}}/C_{\text{ivc}} < 5$ had a low to moderate agreement with the reference samples. The samples with $C_{\text{adrenal}}/C_{\text{ivc}} 1.1-1.99$, $2-2.99$ and $3-4.99$ showed concordant assessments in 39, 52 and 72% of cases with respect to the presence or absence of lateralisation of aldosterone secretion.

The analysed samples with $C_{\text{adrenal}}/C_{\text{ivc}} 5-9.99$ and $C_{\text{adrenal}}/C_{\text{ivc}} \geq 10$ demonstrated the agreement with the reference samples in 85 and 94% of cases respectively. Table 4 shows the detailed data of the analysed samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 5$, which led to the discordant assessment in the identification of lateralisation of aldosterone secretion when compared with their reference samples.

The agreement between the analysed and reference samples was not changed when the ACR cut-off was changed to > 3 and > 5 , as shown in Table 5.

Implementing the suppression of the contralateral adrenal gland ($\text{AC}_{\text{non-dominant adrenal}} < \text{AC}_{\text{ivc}}$) in the definition of lateralisation of aldosterone secretion did not influence the level of agreement.

Discussion

Our study has confirmed that the relative cortisol concentration of adrenal samples significantly impacts the interpretation of AVS data. As expected, the best results were observed from samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 10$; however, even in this group of samples, the agreement in the identification of lateralisation of aldosterone secretion was not absolute. Therefore, clinicians can never be completely free of doubt when deciding about adrenalectomy, even when using samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 10$ for the interpretation of AVS data.

Despite this limitation, AVS based on samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 10$ can be considered as the best approach (gold standard) for the preoperative identification of patients with excessive unilateral aldosterone. We do not suppose that samples with even higher $C_{\text{adrenal}}/C_{\text{ivc}}$ are characterised by a better agreement, as suggested in Table 4 (samples 8–11).

Although adrenal samples with $C_{\text{adrenal}}/C_{\text{ivc}} 5-9.99$ might have a slightly higher risk of erroneous decisions about adrenalectomy, we still consider these acceptable from a clinical point of view.

Table 3 The comparison of analysed and reference samples in the detection of abnormal lateralisation of aldosterone secretion.

	Analysed samples				
	$C_{\text{adrenal}}/C_{\text{ivc}} 1.1-1.99$ ($n=44$)	$C_{\text{adrenal}}/C_{\text{ivc}} 2-2.99$ ($n=25$)	$C_{\text{adrenal}}/C_{\text{ivc}} 3-4.99$ ($n=25$)	$C_{\text{adrenal}}/C_{\text{ivc}} 5-9.99$ ($n=20$)	$C_{\text{adrenal}}/C_{\text{ivc}} \geq 10$ ($n=111$)
Concordant assessment compared to reference samples	17 (39%)	13 (52%)	18 (72%)	17 (85%)	104 (94%)
Lateralisation of aldosterone secretion (from the left adrenal)	5 (3)	7 (5)	3 (2)	12 (5)	42 (18)
Bilateral secretion of aldosterone from both adrenals	12	6	15	5	62
Discordant assessment compared to reference samples	27 (61%)	12 (48%)	7 (28%)	3 (15%)	7 (6%)
Unrecognised lateralisation of aldosterone secretion (from the left adrenal)	11 (7)	4 (1)	2 (2)	2 (2)	6 (4)
False lateralisation of aldosterone secretion (from the left adrenal)	15 (15)	7 (6)	5 (4)	1 (1)	1 (0)
Side interchange (false lateralisation of aldosterone secretion from the left adrenal)	1	1	0	0	0

Values are expressed as the number of samples (and percentages). $C_{\text{adrenal}}/C_{\text{ivc}}$ represents the ratio of cortisol concentrations in the adrenal vein and in the inferior vena cava. n denotes the number of analysed samples.

Table 4 Analysed samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 5$ that led to discrepancies between the ACR_{analysed} and the $ACR_{\text{reference}}$.

	Side	Analysed samples		Ipsilateral reference samples		Contralateral reference samples		Lateralisation of aldosterone secretion	
		$C_{\text{adrenal}}/C_{\text{ivc}}$	AC	$C_{\text{adrenal}}/C_{\text{ivc}}$	AC	$C_{\text{adrenal}}/C_{\text{ivc}}$	AC	ACR_{analysed}	$ACR_{\text{reference}}$
1	R	6.19	1.27	23.71	4.21	29.90	6.37	5.03	1.51
2	R	8.50	2.18	72.50	3.41	70.00	9.29	4.27	2.72
3	R	9.55	6.08	15.45	6.73	19.09	1.57	3.87	4.28
4	R	14.77	3.69	20.45	2.56	10.23	10.44	1.14	4.09
5	R	15.66	3.16	18.07	2.66	19.28	11.27	3.56	4.24
6	R	16.67	2.69	17.95	1.07	42.31	12.00	4.46	11.20
7	R	17.05	3.93	20.45	2.56	10.23	10.44	1.31	4.09
8	L	20.00	0.66	21.50	0.39	13.00	1.68	2.54	4.26
9	R	35.71	46.30	53.57	9.17	15.71	2.49	18.60	3.68
10	R	37.86	1.98	44.17	1.86	29.17	7.47	3.77	4.02
11	R	38.03	1.93	42.25	5.30	23.94	0.64	3.00	8.27

These samples represent those where there was a significant difference between the ACR_{analysed} and the $ACR_{\text{reference}}$ and/or discordant assessment with respect to the lateralisation of aldosterone secretion. Abnormal lateralisation of aldosterone secretion was defined as $ACR > 4$. Reference samples were defined by the highest $C_{\text{adrenal}}/C_{\text{ivc}}$ achieved in a given adrenal. Ipsilateral and contralateral reflects the relation to the analysed samples. Values are expressed as the ratio of hormonal concentrations. AC represents the cortisol-corrected aldosterone concentration, defined as a ratio of aldosterone (pmol/l) and cortisol (nmol/l) in an adrenal sample. ACR_{analysed} represents the ratio of ACs based on the analysed and contralateral reference samples. $ACR_{\text{reference}}$ represents the ratio of ACs of both adrenals in a given individual. $C_{\text{adrenal}}/C_{\text{ivc}}$ represents the ratio of cortisol concentrations in the adrenal vein and in the inferior vena cava. L and R represent left and right adrenal gland.

When analysing the samples with $C_{\text{adrenal}}/C_{\text{ivc}}$ 3–4.99, we observed only moderate agreement with their reference samples. Moreover, this group of samples exhibited a low correlation of cortisol-corrected ACs when compared with the reference samples. If the samples with $C_{\text{adrenal}}/C_{\text{ivc}}$ 3–4.99 had been used for the interpretation of AVS data, this would have resulted in a high number of inappropriately performed adrenalectomies (Table 3). Therefore, we cannot accept these samples for clinical decision making. We can only speculate whether a subgroup characterised by $C_{\text{adrenal}}/C_{\text{ivc}}$ 4–4.99 would have led to a better agreement. Unfortunately, our data contained only a small number of these samples, and did not enable us to perform a more precise analysis. The samples with low relative cortisol concentrations ($C_{\text{adrenal}}/C_{\text{ivc}} < 3$) do not represent the secretion of the evaluated adrenal glands. The agreement with the respective reference samples was low, and the side interchange in the identification of lateralisation of aldosterone secretion was noted.

There are several factors that may explain the discordant results between the analysed and reference samples. First, excessive dilution of adrenal venous

blood resulting from improper adrenal sampling, principally, may have influenced the hormonal concentrations in a significant number of analysed samples. Actually, 96% of the analysed samples with $C_{\text{adrenal}}/C_{\text{ivc}}$ 1.1–4.99 were taken from the right adrenal veins, which are more difficult to cannulate (16). This argument supports the fact that the analysed samples with $C_{\text{adrenal}}/C_{\text{ivc}}$ 1.1–4.99 frequently falsely 'shifted' the aldosterone secretion towards the left adrenal gland, as demonstrated in Table 3.

Secondly, the inherent impreciseness of laboratory estimations of hormonal concentrations may have contributed to the observed discrepancies in samples with both low and high relative cortisol concentrations. The precision of laboratory tests describes a CV that is defined by the ratio of the s.d. to the mean. According to these statistics, 68.3% of values measured from one sample are within the range of one s.d. from the mean. CVs of 5% for cortisol and 7% for aldosterone mean that 31.7% of the measured values from one sample lie more than ± 5 and $\pm 7\%$ away from the mean. Consequently, it is likely that the estimated cortisol concentrations in two non-adrenal samples could differ by more than 10%. This fact

Table 5 The agreement between the analysed and reference samples in the detection of lateralisation of aldosterone secretion depending upon different ACR cut-offs.

ACR cut-off	$C_{\text{adrenal}}/C_{\text{ivc}}$ 1.1–1.99 (%) ($n=44$)	$C_{\text{adrenal}}/C_{\text{ivc}}$ 2–2.99 (%) ($n=25$)	$C_{\text{adrenal}}/C_{\text{ivc}}$ 3–4.99 (%) ($n=25$)	$C_{\text{adrenal}}/C_{\text{ivc}}$ 5–9.99 (%) ($n=20$)	$C_{\text{adrenal}}/C_{\text{ivc}} \geq 10$ (%) ($n=111$)
> 3	36	60	44	90	92
> 4	39	52	72	85	94
> 5	45	36	76	90	94

ACR represents the ratio of ACs from both adrenals, defined as: $AC_{\text{dominant adrenal}}/AC_{\text{non-dominant adrenal}}$. AC represents the cortisol-corrected aldosterone concentration, defined as a ratio of the aldosterone and cortisol concentrations in an adrenal sample. $C_{\text{adrenal}}/C_{\text{ivc}}$ represents the ratio of the cortisol concentrations in the adrenal vein and in the inferior vena cava. n denotes the number of analysed samples.

might explain the enormous number of discrepancies observed in samples with $C_{\text{adrenal}}/C_{\text{ivc}} 1.1\text{--}1.99$. The inherent imprecision of laboratory measurements might be amplified when assessing the lateralisation of aldosterone secretion since ACR is derived from four estimations of hormonal concentrations. Another potential source of laboratory-related error lies in the need to dilute samples when the hormonal concentrations exceed the upper detection limit of the assay used.

Thirdly, superselective adrenal sampling can occur in adrenals with aldosterone-producing adenomas. When the blood is not drawn from the principal adrenal vein that drains the entire gland, but is instead drawn from one of its branches (or an accessory adrenal vein) that selectively drains an adenoma or unaffected adrenal tissue, discrepancies in AC can be noted among high $C_{\text{adrenal}}/C_{\text{ivc}}$ samples taken from one adrenal. According to our experience, this phenomenon is probably rare. One possible example of superselective sampling is shown in Table 4 (sample 9). The data describes a patient with a right-sided Conn's adenoma that was confirmed by marked clinical improvement after surgery. In the analysed sample, there was a markedly higher cortisol-corrected AC as compared with the reference sample containing a higher concentration of cortisol. This finding suggests that the analysed sample was taken from the branch of the adrenal vein that predominantly drained the aldosterone-producing adenoma.

Fourthly, we cannot definitely exclude small fluctuations in hormonal concentrations during sequential adrenal sampling despite the fact that all AVS procedures were performed under highly supraphysiologic cosyntrophin infusion.

The main limitations of this study are the retrospective design and the fact that our results are related uniquely to the AVS procedures performed under cosyntrophin infusion. It is not clear to what extent our data are applicable to the AVS protocols that do not use adrenal stimulation.

To the best of our knowledge, similar data have not previously been published. There are only a limited number of reports that assess the various AVS interpretation criteria with respect to the clinical effect after unilateral adrenalectomy. In 2001, Rossi described that $C_{\text{adrenal}}/C_{\text{ivc}} \geq 1.1$ is an optimal cut-off for selective adrenal sampling (14); however, this report was based on AVS procedures performed without adrenal stimulation by cosyntrophin. In fact, in this study, $C_{\text{adrenal}}/C_{\text{ivc}} \geq 2$ was the highest evaluated cut-off value to identify selective adrenal samples. Moreover, the method for estimating the optimal cut-off was diametrically different from the designs of our analysis. We are not able to compare their findings with our results. In 2008, the same group described increased selectivity of adrenal sampling when performed with cosyntrophin infusion (17); however, no incremental diagnostic value

was observed in $C_{\text{adrenal}}/C_{\text{ivc}}$ cut-off ≥ 2 , which contrasts to our observations. A recent retrospective analysis by Kline *et al.* (10) described the discrepancies among various criteria for the interpretation of AVS results, and raised doubts about the validity of AVS; however, it is important to note that $C_{\text{adrenal}}/C_{\text{ivc}} \geq 5$ was achieved in only 40% of the evaluated AVSs in this report.

Contrary to previously mentioned reports, neither the optimal lateralisation index (ACR) nor the clinical outcome of patients after adrenalectomy was assessed in our analysis. The only aim of our study was to evaluate the quality and consistency of information regarding secretion of adrenal hormones obtained from separate adrenal samples with different cortisol concentrations taken during one session. Nevertheless, we consider our data very important for routine clinical practices. These data show that adrenal samples are acceptable for clinical decision making when they are characterised by $C_{\text{adrenal}}/C_{\text{ivc}} \geq 5$. The AVS results might be better when multiple samples with $C_{\text{adrenal}}/C_{\text{ivc}} \geq 10$ are obtained from both adrenal glands.

Conclusion

Our study clearly demonstrates that AVS provides consistent information when adrenal samples with high cortisol concentrations are used for the interpretation of the data.

Declaration of interest

There are no pending conflicts of interest.

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