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**A case of adrenal vein sampling in primary aldosteronism with homolateral suppression**

**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1742450> since 2020-06-29T12:53:51Z

*Published version:*

DOI:10.1210/js.2016-1105

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(Article begins on next page)

1 **Supplemental File**

2 **A CASE OF ADRENAL VEIN SAMPLING IN PRIMARY ALDOSTERONISM WITH**  
3 **“HOMOLATERAL SUPPRESSION”**

4  
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18  
19 Word count: abstract 233; text 1472 excluding references, legends and figure captions.

20 Number of Tables: 2; Number of Figures: 2; Supplemental file: 1.

21 Running title: adrenal sampling in primary aldosteronism

22 Keywords: aldosterone producing adenoma; adrenal venous sampling; cosyntropin stimulation;  
23 aldosterone, endocrine hypertension.

24  
25 Source of founding: none.

26 Financial disclosure: none.

1 **Immunohistochemistry**

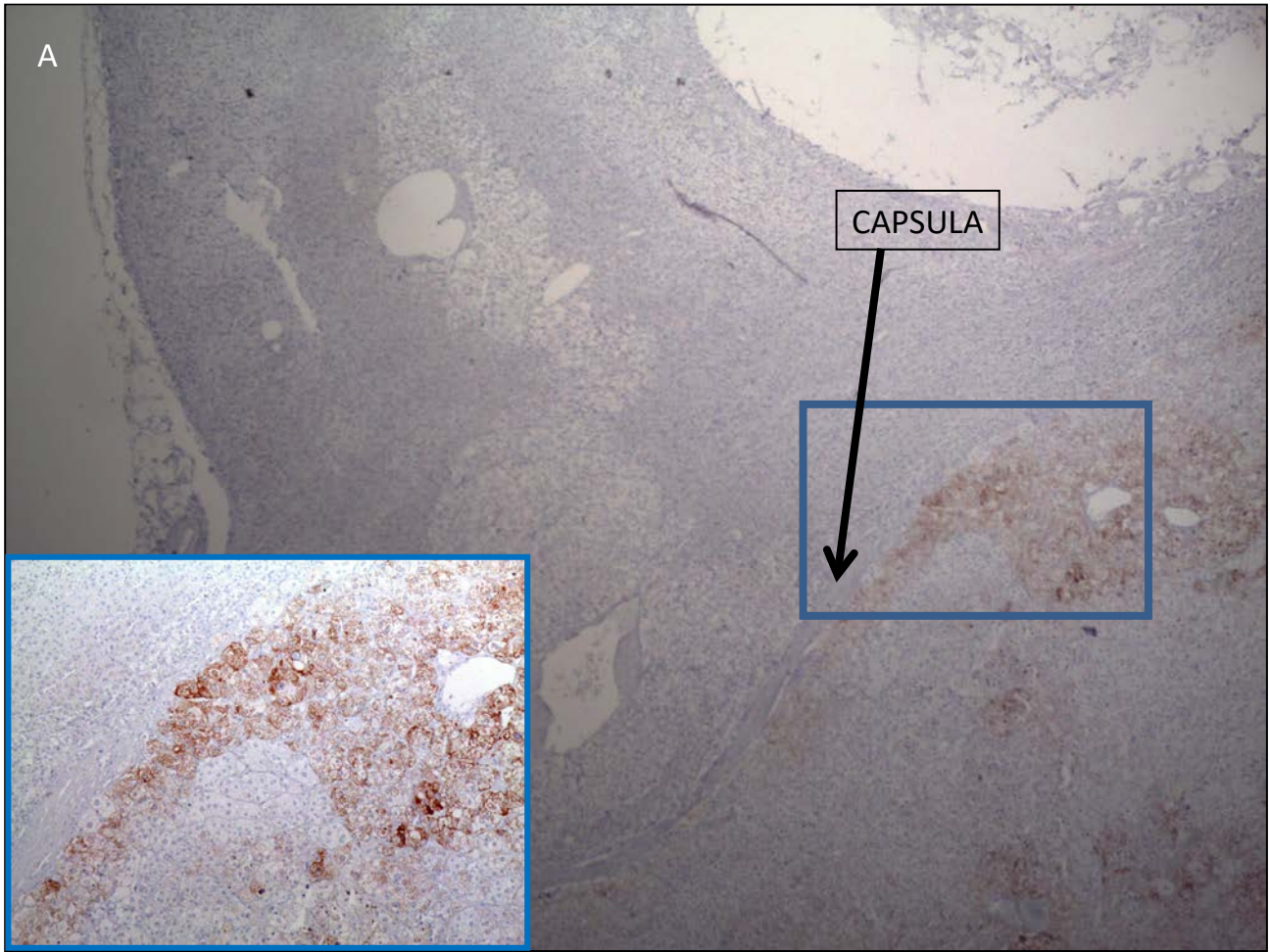
2 Sections of 10 µm thickness from paraffin-embedded adrenal tissue of the patient with APA were  
3 quenched for endogenous peroxidase using H<sub>2</sub>O<sub>2</sub> and subsequently incubated with antibodies  
4 against human CYP11B1 (11β-hydroxylase, 1:1000 diluted) or human CYP11B2 (aldosterone  
5 synthase, 1:100 diluted) for 1 h. After rinsing, the EnVision reagent (Dako, Carpinteria, CA)  
6 coupled with peroxidase-labelled polymer was incubated as secondary antibody for 30 min. The  
7 proteins were visualized using 3,3'-diaminobenzidine tetrahydrochloride counterstained with  
8 haematoxylin. Both antibodies were kindly provided by prof. Celso Gomez-Sanchez (University of  
9 Mississippi, Jackson, MS).

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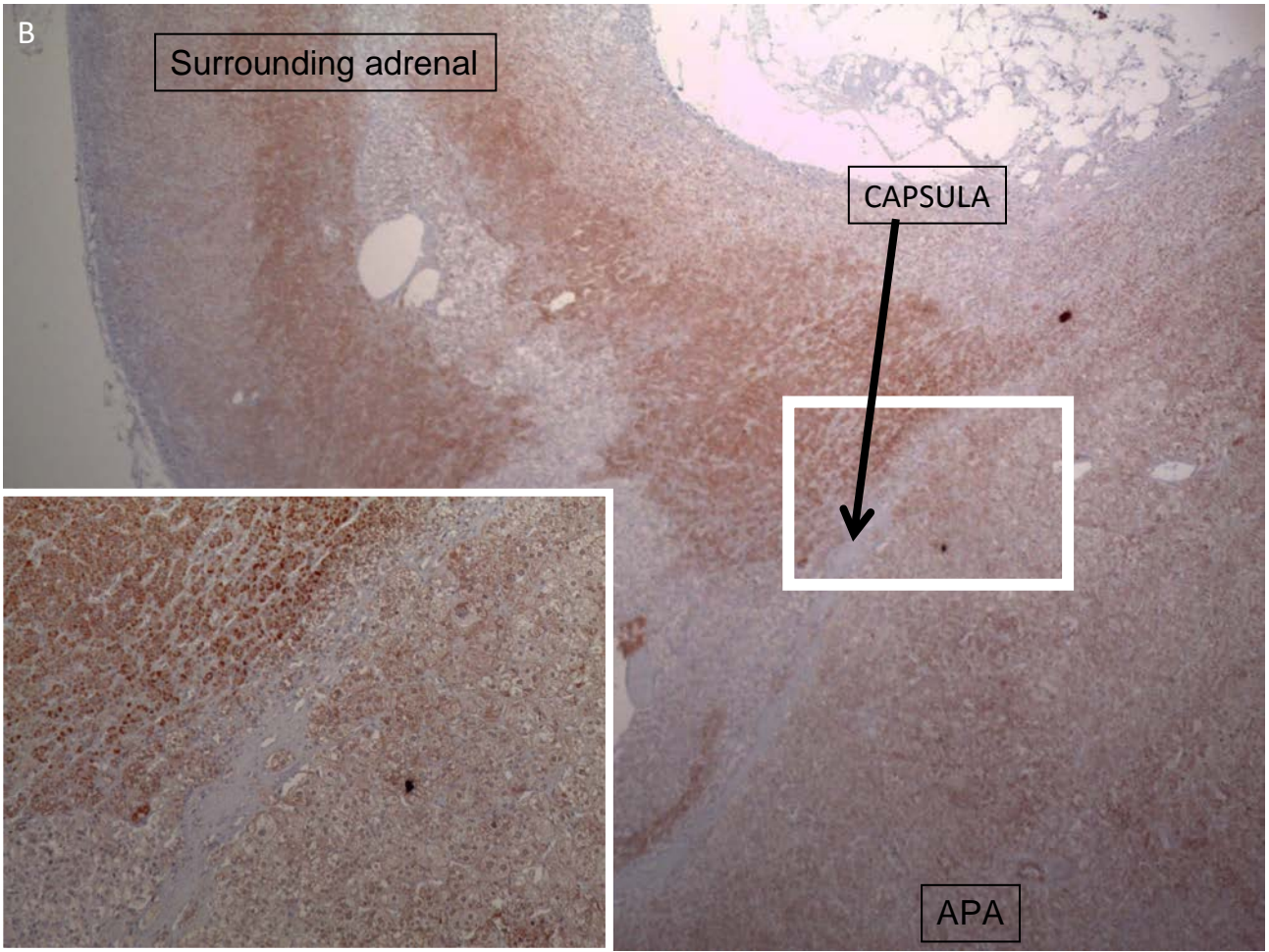
11 **Supplemental figure S1.**

12 Sections of adrenal tissue containing the aldosterone-producing adenoma (APA) and the  
13 surrounding adrenal tissue were immuno-stained with antibodies against human aldosterone  
14 synthase (CYP11B2) (**panel A**) and human 11β-hydroxylase (CYP11B1) (**panel B**).

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<b>Unstimulated AVS</b>	<b>Routine Cortisol (µg/dl)</b>	<b>Aldosterone (ng/dl)</b>	<b>SI</b>	<b>ACR</b>
Infra-renal inferior vena cava	10.3	39.6	-	3.8
Left adrenal vein	65.1	78.2	6.3	1.2
Right adrenal vein – Sample 1 (APCC)	244	251	23.7	1
Right adrenal vein – Sample 2 (APA)	269	5310	26.1	19.7
Right adrenal vein – Sample 3 (central vein)	210	3767	20.4	17.9
<b>Cosyntropin-stimulated AVS</b>	<b>Routine Cortisol (µg/dl)</b>	<b>Aldosterone (ng/dl)</b>	<b>SI</b>	<b>ACR</b>
Infra-renal inferior vena cava	29	57.2	-	2
Left adrenal vein	183	1268	8.2	0.7
Right adrenal vein – Sample 1 (APCC)	778	622	26.8	0.8
Right adrenal vein – Sample 2 (APA)	575	6831	19.8	11.9
Right adrenal vein – Sample 3 (central vein)	625	5123	21.6	8.2

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2 **Table S1. Hormonal measurements from adrenal venous sampling of a second case.** AVS=

3 Adrenal Venous Sampling; SI= Selectivity Index; ACR= Aldosterone/Cortisol Ratio; APCC=

4 aldosterone-producing cell cluster.

5 This table refers to a different patient who underwent super-selective AVS: the LI (ACR right/ACR

6 left) from the central adrenal vein is 14.9 during unstimulated AVS and 11.7 during cosyntropin

7 infusion. ACR from the left adrenal vein and from the adrenal branch draining the area where

8 APCC is present are less than ACR from the peripheral vein, both under basal condition and after

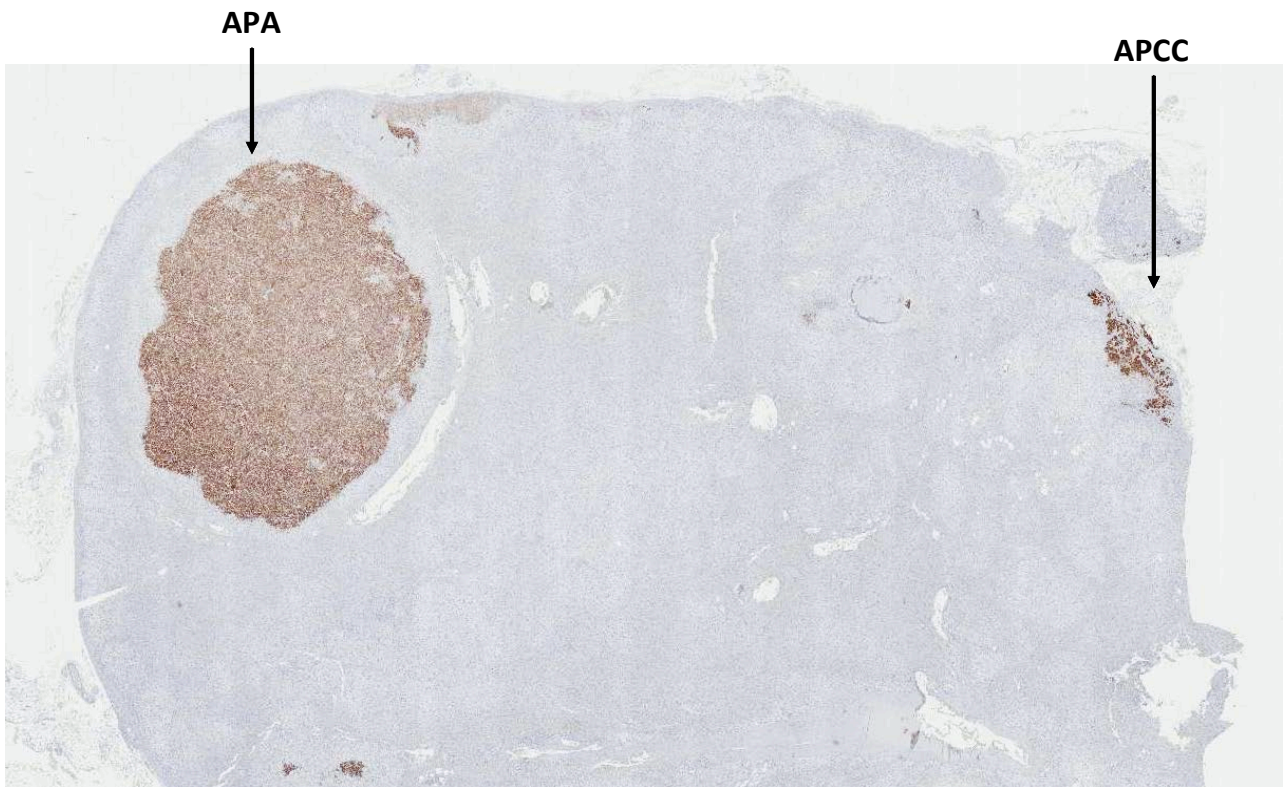
9 cosyntropin infusion. The presence of an APCC outside the main nodule does not affect

10 significantly aldosterone production and ACRs in this patient.



1 **Supplemental figure S2.**

2 Sections of adrenal tissue from patient described in table S1, containing the aldosterone-producing  
3 adenoma (APA) and the surrounding adrenal tissue containing an aldosterone-producing cell cluster  
4 (APCC), immuno-stained with antibodies against human aldosterone synthase (CYP11B2).



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