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# Succinate detection by *in vivo* <sup>1</sup>H-MR spectroscopy identifies germline and somatic *SDHx* mutations in paragangliomas

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## ABSTRACT

### Purpose

Germline mutations in genes encoding succinate dehydrogenase (SDH) are frequent in patients with pheochromocytoma and paraganglioma (PPGL). They lead to SDH inactivation, mediating a massive accumulation of succinate, which constitutes a highly specific biomarker of *SDHx*-mutated tumors when measured *in vitro*. In a recent pilot study, we showed that magnetic resonance spectroscopy (<sup>1</sup>H-MRS) optimized for succinate detection (SUCCES) could detect succinate *in vivo* in both allografted mouse models and PPGL patients. The objective of this study was to prospectively assess the diagnostic performances of <sup>1</sup>H-MRS SUCCES sequence for the identification of SDH deficiency in PPGL patients.

### Methods

Forty-Nine patients presenting with 50 PPGLs were prospectively enrolled in our referral center for <sup>1</sup>H-MRS SUCCES. Two observers blinded to the clinical characteristics and genetic status analyzed the presence of a succinate peak and confronted the results to a composite gold standard combining PPGL genetic testing and/or *in vitro* protein analyses in the tumor.

### Results

A succinate peak was observed in 20 tumors, all of which had proven SDH deficiency using the gold standard (17 patients with a germline *SDHx* mutations, 2 with a somatic *SDHD* mutation and 1 with negative SDHB IHC and SDH loss of function). A false negative result was observed in 3 tumors. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of <sup>1</sup>H-MRS SUCCES were respectively 87%, 100%, 100%, 90% and 94%.

### Conclusions

Detection of succinate by <sup>1</sup>H-MRS is a highly specific and sensitive hallmark of SDH-deficiency in PPGLs.

## INTRODUCTION

Pheochromocytomas and paragangliomas (PPGL) are characterized by a very strong genetic determinism with almost 20 susceptibility genes identified so far, which mutations can impact the prognosis, clinical outcome [1] and response to treatment [2]. More than half of inherited PPGL involve mutations in genes encoding mitochondrial enzymes or transporters. Among them, *SDHx* genes (*SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*) encode the four subunits and the assembly factor of succinate dehydrogenase (SDH), which oxidizes succinate to fumarate in the tricarboxylic acid (TCA) cycle and constitutes the complex II of the electron transport chain. *SDHx* mutations predispose to multiple PPGL and *SDHB* mutated tumors are of strongly increased risk of metastatic progression [3, 4]. While *SDHB* mutations are found in around 10% of PPGL patients, it is estimated that more than one third of patients with metastatic PPGL carry an *SDHB* germline mutation. Similarly, it is estimated that approximately 15% of PPGL patients will ultimately develop a metastatic disease, *versus* 50% of *SDHB*-mutation carriers. Patients with hereditary *SDHx*-related PPGL carry a germline heterozygous mutation, which is associated with the somatic inactivation of the normal allele by somatic mutation or loss of heterozygosity according to the Knudson's 2-hit model for tumor suppressor genes. This bi-allelic inactivation induces a complete loss of activity SDH in the tumor, leading to the accumulation of succinate, which acts as an oncometabolite and is suspected to mediate most tumorigenic effects related to *SDHx* mutations [5-8].

In *SDHx*-mutated PPGL, succinate concentrations increase dramatically, up to 100-fold with respect to tumors without *SDHx* mutations [9-11]. We recently adapted a magnetic resonance spectroscopy (MRS) method to detect succinate in tumors (SUCCinate Estimation by Spectroscopy (SUCCES)) relying on a monovoxel PRESS asymmetric « PROBE » sequence. This pilot study [12] performed in 9 patients with PPGL (5 with *SDHx* mutations and 4 sporadic cases) demonstrated that succinate accumulation was indeed detectable in the tumors of all *SDHx*-mutated patients but not in non-*SDHx*

1 tumors. An unexplained choline peak was also observed in *SDHx*-mutated cases. These results  
2 supported the proof-of-concept that noninvasive detection of succinate by *in vivo* <sup>1</sup>H-MRS may  
3 constitute a specific biomarker of *SDHx* mutations. Following this first-in-man study, we here report  
4 the results of a prospective study aiming at evaluating the clinical relevance of <sup>1</sup>H-MRS SUCCES in  
5 the routine care of PPGL patients.  
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## 10 MATERIALS AND METHODS

### 11 Patients

12 Patients explored for a PPGL in the hypertension unit of Hôpital Européen Georges Pompidou, Paris  
13 (France) were included prospectively if they met the following criteria: 1- age ≥ 18 years-old; 2- PPGL  
14 diagnosis in accordance with international clinical practice guidelines [in a reference centre, relying](#)  
15 [on biochemistry, cross-sectional and functional imaging \[13\]](#); 3- signed informed consent for PPGL  
16 genetic testing; 4- no contraindications to MRI (i.e metallic implants, claustrophobia,...).  
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35 Ethical approval for the study was obtained from the institutional review board (Comité de  
36 Protection des Personnes (CPP) Ile de France II). All patients provided written informed consent for  
37 participation to the study, collection of samples and subsequent analyses.  
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52 When a surgical procedure was performed, fresh tumor samples were frozen immediately after  
53 surgical resection and subsequently stored at -80°C until processing following the COMETE collection  
54 procedures [14]. Histologic diagnosis was performed on paraffin-embedded formalin fixed samples.  
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### 66 Succinate detection by <sup>1</sup>H-MRS at 3 Tesla

67 Combined MR images and MR spectroscopic scans of patients were acquired in a 3 Tesla MRI clinical  
68 scanner (Discovery MR750w GEMSOW, GE Medical Systems, Milwaukee, WI), as previously  
69 described [12]. <sup>1</sup>H-MRS spectra were acquired using the optimized SUCCES sequence: asymmetric  
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Point REsolved SpectroScopy (PRESS) monovoxel acquisition based on the PROBE monovoxel sequence[15], with TR: 2500 ms; TE: 144 ms; Nex: 512 (22 min acquisition) or 1024 (44 min acquisition).

Detection of tumors and VOI (Volume of Interest) positioning were performed on thin-section high-resolution T2-weighted fast spin-echo imaging in at least two orthogonal planes with the following parameters: TR: 2500 ms; TE: 85 ms; echo train length: 19; slice thickness: 2 mm; spacing: 0.3; field of view: 14x14cm for neck or 42x42 cm for whole body coil; matrix: 320x320 or on thin-section high-resolution balanced steady-state gradient echo sequence (*FIESTA*) in at least two orthogonal planes with the following parameters: TR: 4.9 ms; TE: 1.4 ms; NEX:1; Flip angle : 50; slice thickness: 3 mm; spacing: 1; field of view: 14x14cm for neck or 42x42 cm for whole body coil; matrix: 256x256.

### **Immunohistochemistry**

SDHA and SDHB protein expression were assessed on formalin-fixed paraffin embedded (FFPE) tumor samples by immunohistochemistry (IHC) as previously described [16-18] using the following antibodies and conditions: anti-SDHA (ab14715, Abcam; 1:1000) and anti-SDHB (HPA002868, Sigma-Aldrich Corp; 1:500) .

### **SDH activity**

SDH activity was investigated, when necessary, on frozen tumor samples, homogenized using a 1 ml glass-glass Potter-Elvehjem, using a spectrophotometric assay, as previously described[19].

### **Genetic analysis**

DNA extracted from leukocytes was genotyped in all included patients, as well as DNA extracted from frozen or FFPE-tumors when available. Genetic analyses were performed by Next Generation Sequencing (NGS) using the 'MASTR plus SDHv2' targeted panel (Multiplicom, Agilent technologies)

as previously reported and in accordance with international guidelines [20, 21].

## Data analysis

Two independent investigators (CLL and ABE), blinded from clinical observations and genetic test results at the time of analyses, examined all spectra in a qualitative manner and scored for the presence or absence of a succinate peak at 2.44ppm, lactate/lipid signal (0.9 to 1.3 ppm), choline peak (3.2 ppm). When spectra interpretation was different between the two investigators, discordance(s) have been resolved by consensus by a panel of experts (CLL, ABE, JF and BT). Tumor and VOI size as well as tumor heterogeneity were evaluated on the FIESTA or the T2-weighted fast spin-echo sequence.

<sup>1</sup>H-MRS data were initially compared to the genetic gold standard of SDH deficiency assessed by germline genetic testing. In cases of discrepancies between germline genetic test and <sup>1</sup>H-MRS results, a composite gold standard was applied by considering the results of tumor tissue analyses (somatic NGS, SDHB IHC or SDH activity measurement in the tumor). Tumors were thus considered as SDH deficient when (i) an *SDHx* mutation was detected on germline and/or (ii) tumor DNAs, or (iii) when SDH inactivation was supported by both of the protein analyses of tissue samples (SDHB/A negative immunohistochemistry and loss of SDH activity).

Sensitivity (Se), specificity (Sp), positive/ negative predictive values (PPV/NPV) and accuracy of the method were calculated using the SISA (Simple Interactive Statistical Analysis) procedure with continuity corrected Wilson 95% confidence intervals presentation.

## RESULTS

### Patients

Fifty-six patients, including the 9 from the original pilot study [12], were prospectively enrolled between January 2015 and March 2019, leading to the exploration of 57 tumors with the <sup>1</sup>H-MRS SUCCES sequence (one abdominal PGL and one head and neck PGL were explored in patient 1 (previously reported in the pilot study, Table S1) carrying an *SDHB* mutation [12]). Seven patients were excluded. One presented a surgical clip, one a non-evaluable highly necrotic tumor, one was diagnosed with a poorly differentiated neuroendocrine tumor instead of a PPGL following pathological analysis of the tumor and the “gold standard” data could not be obtained for four of them. Overall, 49 patients with 50 tumors were analyzed (Figure 1) and their main characteristics are presented in Table 1. Median age at the time of <sup>1</sup>H-MRS examination was 44.7 years-old [20-75]. The 50 tumors were located as follow: 24 head and neck PGL, 9 abdominal PGL and 17 pheochromocytomas (PCC).

### <sup>1</sup>H-MRS SUCCES performance and added value for management of patients

A succinate peak at 2.44ppm was observed in 20 tumors (Figure 2), while a choline peak was detected in 27 tumors, including 17 with an associated succinate peak. Genetic status was known at the time of <sup>1</sup>H-MRS for 9 patients (18%). For the remaining 40 patients, genetic status was available for clinician after genetic counseling and NGS analyses of germline and somatic DNA in average 12.2 months (± 8.6 months) after <sup>1</sup>H-MRS results. Among the 19 patients (20 tumors) showing a tumor succinate accumulation, 16 (17 tumors) were confirmed to carry a germline *SDHx* mutation: 1 *SDHA*, 4 *SDHB*, 2 *SDHC* and 9 *SDHD*.

In 3 patients, <sup>1</sup>H-MRS SUCCES suggested SDH deficiency despite a negative genetic test. In 2 of them, NGS analysis of tumor DNA identified an *SDHD* somatic mutation (patients 20 and 21, Table S1). The third patient (Patient 22, Table S1) was a forty-two year-old patient with a familial history PPGL (his



1 father developed bilateral carotid body PGL diagnosed at 40 years-old). The patient suffered from a  
2 right vagal PGL operated ten years earlier and a left vagal PGL treated at the same period by external  
3 beam radiotherapy. <sup>1</sup>H-MRS SUCCES was performed in a metastatic relapse of the left vagal PGL and  
4 showed a succinate peak. PPGL genetic testing was negative both in germline and somatic DNA, but  
5 SDH deficiency was demonstrated by negative SDHB IHC and complete inhibition of SDH enzymatic  
6 activity in the tumor (Figure 4). Hence, there was no false positive finding.

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14 Thirty tumors (30 patients) showed no succinate accumulation detectable by <sup>1</sup>H-MRS SUCCES. Gold  
15 standard confirmed the absence of SDH deficiency in 27 cases (Table S2). Two patients carried a  
16 germline mutation in the *VHL* gene, while germline genotyping was negative in 25 patients. In the  
17 latter cases, NGS identified 10 with somatic mutations (2 in *EPAS1*, 4 in *HRAS*, 3 in *VHL* and 1 in *NF1*  
18 gene).

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26 A false negative result (i.e. no succinate peak in a patient with an *SDHx* mutation) was observed in 3  
27 tumors (Figure 3). The first patient (*SDHB* mutated, Patient 7) presented with a cervical PGL that  
28 included the carotid artery and the jugular vein, preventing the use of the <sup>1</sup>H-MRS SUCCES which had  
29 to be performed on a very small metastatic lymph node (voxel size 0.7 cm), which may have been a  
30 limiting factor. The second patient (Patient 9) carried a germline *SDHD* gene mutation and suffered  
31 from a right cervical PGL with numerous hemorrhagic and necrotic spots (voxel size 2.5 cm<sup>3</sup>). The  
32 third one was a 20 years old patient (Patient 6) followed in a familial context of germline *SDHD* gene  
33 mutation with a small left-sided PCC (voxel size 1.5 cm<sup>3</sup>), for which respiratory motion may have  
34 compromised the result.

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47 Discordance between the 2 investigators (CLL and ABE) were observed for 2 cases (Patients 13 and  
48 36, Table S1 and S2) and resolved by consensus by the panel of experts.

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52 Altogether, at the lesion level, sensitivity, specificity, PPV, NPV and accuracy of <sup>1</sup>H-MRS SUCCES were  
53 respectively of 87% [Wilson CI 59.2-97.4], 100% [Wilson CI 79-99.8], 100% [Wilson CI 72.6-99.8], 90%  
54 [Wilson CI 66.8-98], and 94% [Wilson CI 78.5-98.8].

## DISCUSSION

To our knowledge, this is the first prospective validation study demonstrating high sensitivity and specificity of non-invasive detection of succinate by *in vivo*  $^1\text{H}$ -MRS. Our results qualify the presence of a succinate peak at 2.44 ppm as a specific *bona fide* biomarker of SDH deficiency in patients suffering from PPGL, whatever the *SDHx* gene mutated (*SDHA*, *B*, *C*, *D*).

We clearly show that  $^1\text{H}$ -MRS SUCCES allows early suspicion of *SDH* deficiency in routine clinical practice. Indeed, for almost 80% of patients, results of  $^1\text{H}$ -MRS SUCCES sequence were provided to clinicians before the results of genetic test. A recent multicentric retrospective study demonstrated that early knowledge of genetic status has a positive impact on the management and clinical outcome of patients with a germline *SDHx* mutation [1]. Identification of a succinate peak with  $^1\text{H}$ -MRS SUCCES sequence should therefore accelerate genetic testing in these patients, focusing and facilitating the interpretation of genetic variations in *SDHx* genes provided by NGS. In a patient with initial PPGL diagnosis, early assessment of *SDHx* mutational status may impact patient's management, by taking into account the higher risk of metastasis of *SDHB*-related tumors, or the high rate of multiple cervical PGL for *SDHD*-mutated patients. Finally, early identification of patients with genetic predisposition will also accelerate the familial pre-symptomatic genetic screening and therefore benefit to relatives of index patients.

Importantly, we demonstrate that, in case of negative germline genotyping, the finding of a positive succinate peak using  $^1\text{H}$ -MRS SUCCES should definitively prompt to search for *SDHx* somatic mutations in tumor DNA. In our series, this approach led to the identification of 2 tumors with somatic *SDHD* variants, which have only been very rarely reported in the past [20, 22, 23]. It could also prompt to go deeper in the exploration of some patients like in patient 22, familial case presenting with an SDH deficiency despite the absence of germline and somatic *SDHx* variants. Several hypotheses such as an intronic mutation on germline DNA not detected by NGS analyses, epigenetic mutations in *SDHx* promoters or mutation in a regulator gene of *SDHx* genes expression, are under evaluation to go further in this case by performing a whole genome sequencing.

Moreover, the identification of a succinate peak in a tumor is a major argument to support the pathogenicity of a variant of unknown significance (VUS) in an *SDHx* gene, which is essential to assess the risk of recurrence, to adapt the follow-up and to manage the genetic counseling to relatives [20, 2]. In our cohort, a VUS was detected in 3 patients and classified as deleterious or not (a likely benign VUS in *SDHA* was ruled out, and 2 VUS, 1 on *SDHB* (Patient 1) and 1 on *SDHD* (Patient 14) genes, were eventually classified as pathogenic), based on *in silico* predictions and *SDHB* IHC. In these 3 patients, <sup>1</sup>H-MRS results were concordant with IHC results, suggesting that <sup>1</sup>H-MRS SUCCES is a of useful contribution for patient management, especially whenever the absence of tumor tissue precludes immunohistochemical analyses. Assessing this tumor hallmark *in vivo* in patients with *SDHx*-related tumors will present benefits in other aspects. In a patient carrying a germline *SDHx* variant it could help: i) to classify a suspicious lesion detected during follow-up and ii) to confirm the metastatic development of the disease after the identification of one (or several) dubious lesion(s) in extra-paraganglionic sites on imaging follow-up. *SDHx* mutations also predispose to other types of tumors such as GIST (GastroIntestinal Stromal Tumors), rare cases of renal cell carcinomas and of pituitary adenomas, which are still debated [24-27]. Interestingly, in a recent study, the application of <sup>1</sup>H-MRS was successfully extended to metastasis of PPGL and GIST and permitted to exclude *SDH* deficiency in a pituitary adenomas developed in an *SDHB* mutation carrier [28]. However, it is important to note that in our study, only 2 patients with an *SDHx* mutation were explored for an abdominal PGL. Additional studies should be performed in the future to evaluate <sup>1</sup>H-MRS SUCCES accuracy for this particular location.

Regarding clinical relevance, we suggest to perform <sup>1</sup>H-MRS SUCCES in all patients with a newly discovered PPGL, in patients with negative germline genetic testing in case of multiple tumors and/or a metastatic development of the disease to tailor therapeutic choices, and in patients already identified to carry a germline *SDHx* mutation in case of dubious finding on imaging follow-up. By

contrast, <sup>1</sup>H-MRS SUCCES would not present any relevance in patients with a known mutation on a non-SDHx susceptibility gene.

Imaging of patients with PPGL is crucial in every step of their management and functional imaging using PET tracers provide important information on tumor biology, highly connected with genetic status [29]. Recent development of PET/MR devices would allow to combine functional imaging and <sup>1</sup>H-MRS, providing simultaneously and non-invasively coregistered anatomical, metabolic (i.e. FDG uptake) and metabolomics (succinate accumulation) information on the tumor.

<sup>1</sup>H-MRS SUCCES permitted in this study to assess for the presence (or absence) of a succinate peak in a qualitative manner. However, quantification of succinate *in vivo* with <sup>1</sup>H-MRS is feasible and development of a specific post-processing method allowing assessment of succinate as a quantitative biomarker over the time course of the disease would be of great interest especially for the evaluation of response to treatment.

It should also be stressed that SUCCES has the same limitations as any <sup>1</sup>H-MRS approach, in particular sensitivity in the low millimolar range. Accordingly, its capacity to detect succinate in PPGL lesions is dependent on: (i) the size of the tumor, which is directly related to detection sensitivity: a voxel size smaller than 1 cm<sup>3</sup> is associated with a high risk of failure (Table 2); (ii) the presence of hemorrhagic or necrotic spots, which is a source of technical flaws: a large necrotic area or inclusion of blood vessel within a tumor may discourage exploration by <sup>1</sup>H-MRS SUCCES (Figure 3). Nevertheless, there is still room for progress and in this respect, several adjustments may improve sensitivity and decrease the sequence duration. These include increasing MRI field using the new research-dedicated 7-tesla devices, post-processing of the spectra or respiratory gating for abdominal tumors. Indeed, respiratory gating would be a possible adjustment for abdominal tumors and in particular for PCC but with an increase of total duration of the sequence, a risk of patient discomfort and then motion. Nevertheless, one solution could be to analyse respiratory-gated

1 acquisition combined with an increased MRI field (7 tesla) that could allow reducing averages  
2 number and thus sequence duration.  
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9 In conclusion, using  $^1\text{H}$ -MRS to detect specific tumor phenotypes induced by genetic mutations is  
10 innocuous (no tissue sampling, no injection of radiopharmaceutical or contrast agent are needed)  
11 and precise. Following the initial pilot studies [12, 30, 28], this study crosses the second translational  
12 gap and demonstrates that  $^1\text{H}$ -MRS SUCCES can be performed in a radiology department during  
13 routine clinical practice.  $^1\text{H}$ -MRS improves genetic diagnosis, providing VUS characterization and  
14 guiding geneticists to extend investigations to somatic NGS.  $^1\text{H}$ -MRS SUCCES is ideal for assessing the  
15 presence of succinate repeatedly over the time course of the disease, for clinical surveillance,  
16 postoperative follow-up, and evaluation of treatment efficacy.  
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## **TABLES AND FIGURES**

### **Figure 1: Flowchart**

**Figure 2: <sup>1</sup>H-MRS SUCCES in SDH-deficient tumors.** A succinate peak is observed at 2.44ppm in head and neck paragangliomas from patients carrying germline *SDHD* (A : Patient 11, B: Patient 12 and D: Patient 14 ) or *SDHB* (C : Patient 15) mutations.

**Figure 3: <sup>1</sup>H-MRS SUCCES failures in SDH-deficient tumors.** A) Left panel shows both the cervical PGL including the carotid artery and the jugular vein and a small metastatic lymph node (arrows) developed by a 35 year-old patient carrying a germline *SDHB* gene mutation (Patient 7). Positionning a small voxel of 0.7cm<sup>3</sup> (middle pannel) did not allow revealing a succinate peak (left). B) T2 (left) and T1-weighted MRI (middle) show a right cervical PGL with hemorrhagic and necrotic spots in a 68 year-old patient with a germline *SDHD* gene mutation (Patient 9) and the absence of succinate peak. C) Small left-sided PCC (voxel size 1.5 cm<sup>3</sup>) in a 20 years old patient followed in a familial context of germline *SDHD* gene mutation (Patient 6) without succinate peak.

**Figure 4: Patient 22 with SDH deficiency demonstrated by functional studies.** A) Axial T2-weighted MRI of the left vagal PGL; B) Maximal intensity projection of 18F-FDopa-PET/CT; C) Axial fused Maximal 18F-FDopa-PET/CT; D) 1H-MRS spectrum showing a succinate (Su) peak at 2.44ppm associated with a choline (Ch) and a lipids/lactate (L) peak; E) SDHB immunohistochemistry in a non-SDH PGL show a typical mitochondrial granular staining while F) the patient's tumor displays a weak diffuse SDHB staining frequently observed in SDHD-mutated PGL (ref) and G) a strong SDHA positive labeling. H) Analysis of succinate cytochrome C reductase (SCCR) activity in the patient's tumor compared with a non-SDH and an SDH-mutated PGL confirms loss of SDH activity.

**Table 1: Baseline characteristics of patients (N=49 patients, 50 tumors)**

**Table 2: Comparison between successes and failures of <sup>1</sup>H-MRS SUCCES in patients with an SDH deficiency**

**Table S1: Characteristics of the 22 patients with an SDH deficiency**

**Table S2 : Analyses performed to rule out SDH deficiency in 27 patients**

**Table 1 Baseline characteristics of patients (N=49 patients, 50 tumors)**

<b>Age</b> (years, Median [range])	44.7 [20-75]
<b>Gender</b>	
Female (%)	25 (51)
<b>Location (%)</b>	
Head and neck PGL	24 (48)
Abdominal PGL	9 (18)
Pheochromocytoma	17 (34)
<b>Outcome (%)</b>	
Surgery	31 (62)
External Beam Therapy	8 (16)
Follow-up with out treatment	8 (16)
Unknown	3 (6)

*PGL: paraganglioma, SDH: succinate dehydrogenase, VHL: Von Hippel Lindau*

**Table 2: Characteristics of the 23 tumors with an SDH deficiency according to <sup>1</sup>H-MRS SUCCES results**

	Failures (FN)	Successes (RP)
N (%)		
✓ Patients	3/22 (14%)	19/22(86%)
✓ Tumors	3/23 (13%)	20/23 (87%)
Size (mean ± SD [range])		
✓ Tumor long axis (cm)	2.7 ± 0.4 [2.4-3.2]	4.6 ± 1.6 [2.3-8.7]
✓ Tumor volume (cm <sup>3</sup> )	6.7 ± 3.3 [3.4-10]	23.3 ± 23.5 [1.6-110]
✓ Voxel volume (cm <sup>3</sup> )	1.7 ± 0.9 [0.7-2.5]	4.6 ± 4.3 [1.2-19.2]
Location		
✓ H&N PGL	2	18
✓ Abdo PGL and PCC	1	2
Necrosis	2/3 (67%)	5/20 (25%)
SDHx mutation		
✓ SDHA	0	1
✓ SDHB	1	4
✓ SDHC	0	2
✓ SDHD	2	9
✓ Somatic SDHD	0	2
✓ Unknown	0	1
Averages		
✓ 512	1 (33%)	5/20 (25%)
✓ 1024	2 (67%)	15/20 (75%)

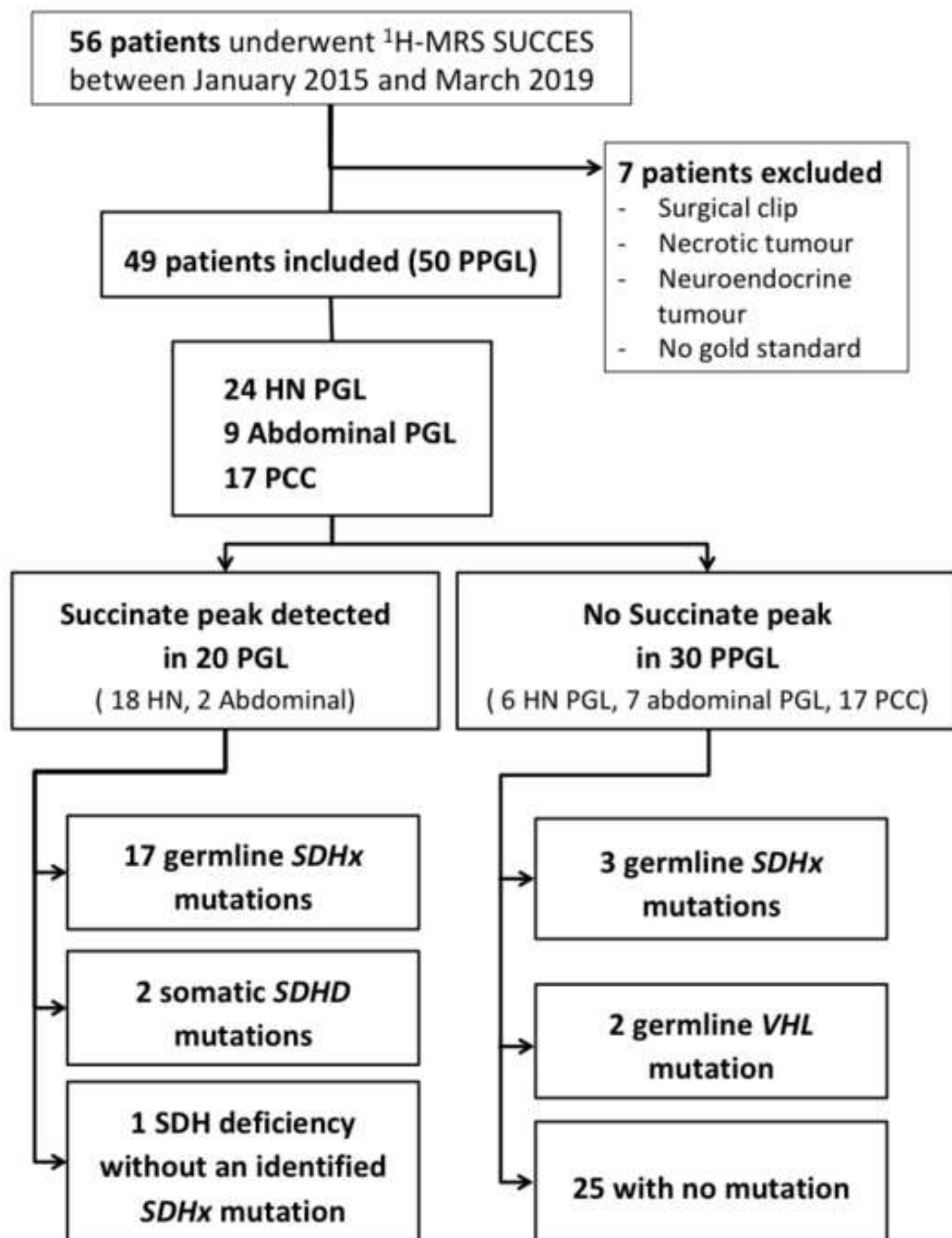


Figure 2

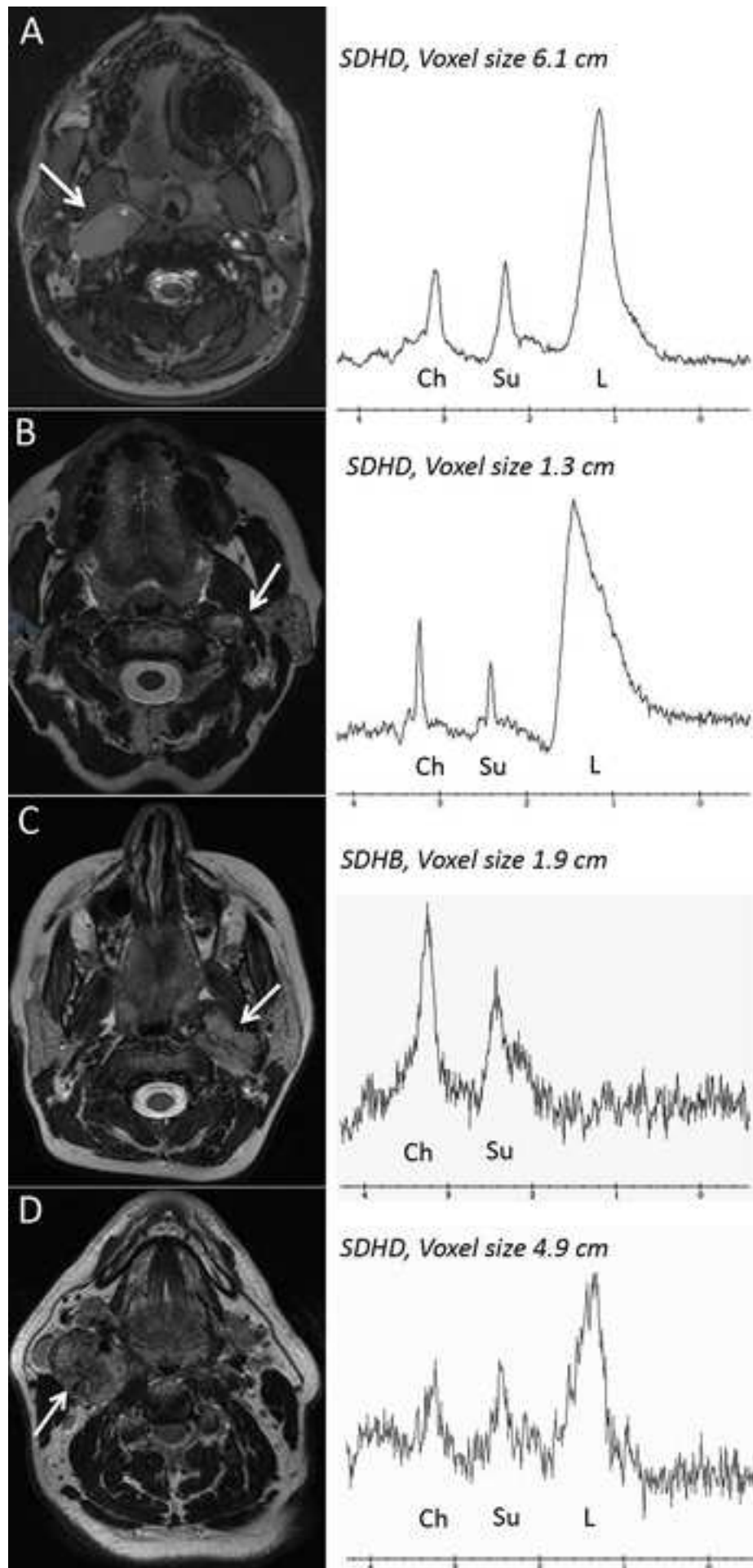


Figure 3

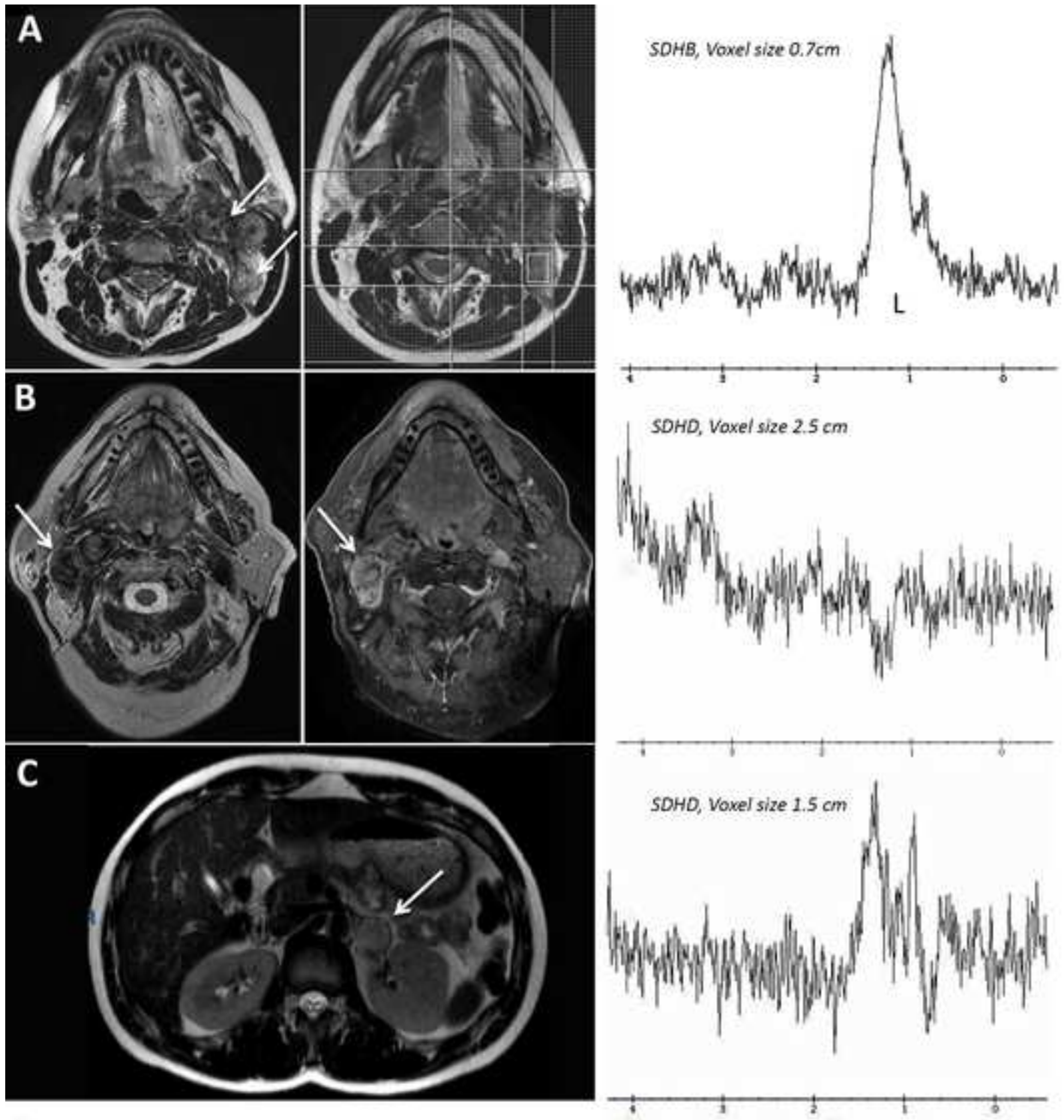
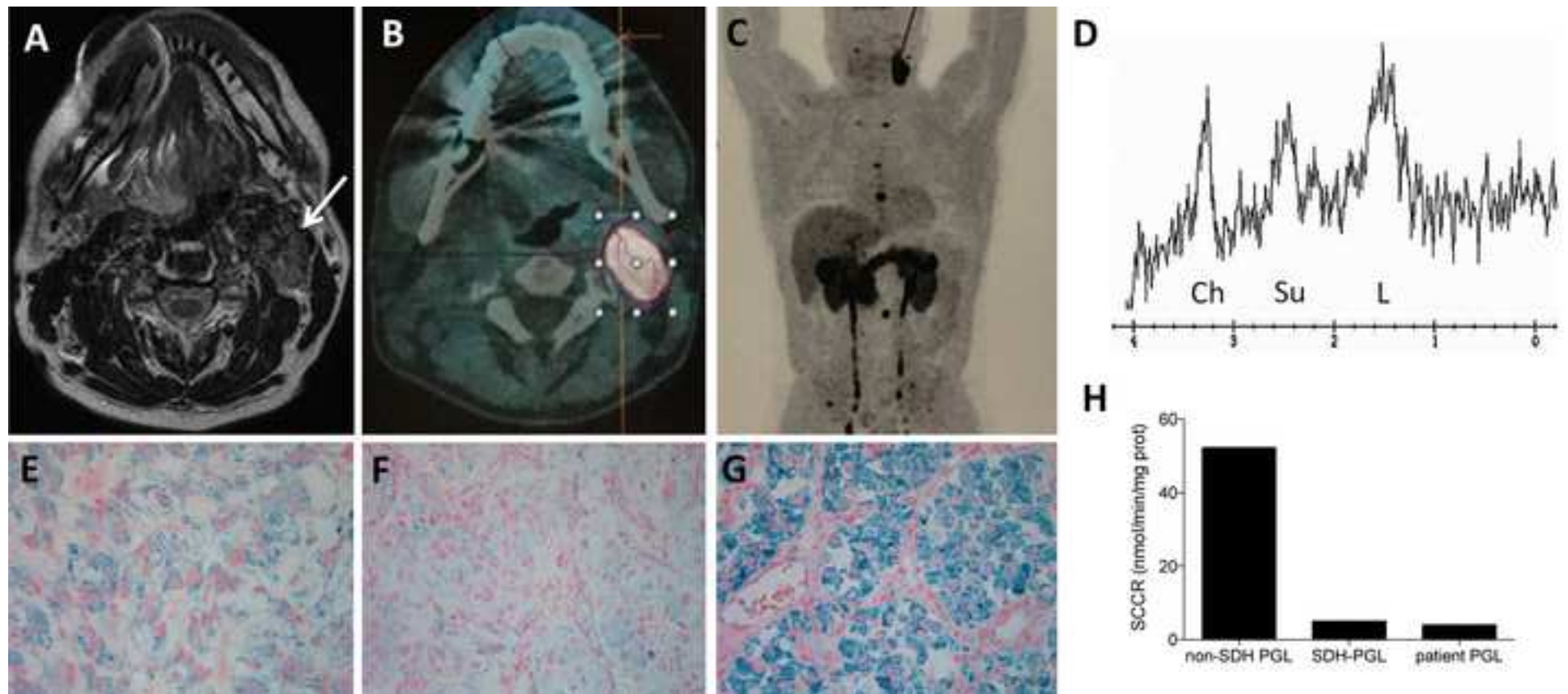




Figure 4





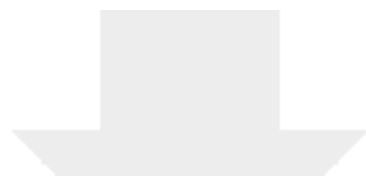


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**Supplementary Material**

Lussey-Lepoutre-Table S1.docx





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**Supplementary Material**

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