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Role of flow magnetic resonance imaging in the monitoring of facial allotransplantations: preliminary results on graft vasculopathy

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Abstract

Chronic vascular rejection characterized by the myointimal proliferation of smooth muscle cells that progressively obstruct the arterial graft lumen may become the main cause of long-term graft loss in vascularized composite allotransplantation (VCA), as observed in solid organ transplantation. As such, new diagnostic tools are required.

The objective of this study was to evaluate the usefulness of flow magnetic resonance imaging (MRI) in the qualitative and quantitative monitoring of VCA in three patients transplanted between 2005 and 2012. Seven flow MRI acquisitions were performed concurrently with standardized clinical and histological monitoring between 2015 and 2017 [Au?1]. A progressive reduction in the average flow rate and intraluminal diameter of the arterial pedicle of the grafts was demonstrated. During follow-up, two patients developed chronic vascular rejection requiring partial resection of the graft. For these patients, flow MRI acquisitions were characterized by a significant reduction in vascular signal, with a reduction in intravascular flow prior to anatomical injury. The results of this study confirm the feasibility of reproducible, non-invasive, and non-operator-dependent morphometric and haemodynamic radiological analysis, providing clinicians with new information on the vascular status of VCA over time and offering the prospect of an imaging technique specific to vascular outflow.

Key words: vascularized composite allotransplantation, magnetic resonance imaging, graft rejection, follow-up studies, haemodynamics, arteries

Introduction

After more than 60 years of experience, chronic graft rejection remains one of the major limitations of long-term organ transplantation. With renal transplantation, in addition to the non-immunological aetiologies of graft loss (e.g. toxicity of calcineurin inhibitors, arterial hypertension, dyslipidemia), the first cause of long-term graft loss is related to humoral rejection¹. Clinically, patients develop changes of vascular rejection, characterized by progressive obliteration of the graft arteries, associated with myointimal proliferation of smooth muscle cells progressively obstructing the arterial lumen, resulting in long-term ischemia and partial or complete loss of the transplant^{2,3}.

Recently, these vascular lesions have also been diagnosed in patients with vascularized composite tissue allotransplantations (VCA). They were probably responsible for partial necrosis of the facial transplant in the first patient grafted in 2005, who developed chronic humoral rejection of the face with typical lesions of allograft vasculopathy⁴. These changes have also been found in upper limb transplants⁵, and have been associated with graft loss in at least five patients⁶.

Since 1986 it has been possible, due to the techniques of flow magnetic resonance imaging (MRI) without injection of contrast medium, to obtain images whose signal depends on both the direction and the velocity of flow⁷⁻⁹. Developed initially for the study of large calibre vessels¹⁰ and then for the study of cerebrospinal fluid^{11,12}, the development and optimization of specific sequences dedicated to infra-centimetric [Au?2] vessels^{13,14} now offers the prospect of a qualitative, quantitative, standardized, and non-invasive analysis of small vessels, such as the branches of the external carotid artery (superior thyroid, lingual, facial, internal maxillary, and

superficial temporal arteries). The aim of this study was to evaluate the potential for longitudinal follow-up of arterial vascularization of facial allotransplants by flow MRI, and to extract the first haemodynamic and morphometric measurements of vascular capital according to a reproducible and non-invasive standardized protocol.

Materials and methods

Flow MRI acquisitions were performed between December 2014 and December 2017 in three patients who had undergone face allotransplantation in Amiens University Hospital [Au?3].

3 Tesla MRI acquisition procedure

All acquisitions were performed on a 3 Tesla research MRI instrument combining a 32-element head coil and a 47-mm diameter microscopic coil. First, a phase contrast three-dimensional angiographic sequence (3D PCA) with an encoding velocity of 30 cm/s (V30) was performed with the 32-element head coil, allowing qualitative analysis of the vascular capital. The encoding speed was defined according to the physiological database of flow¹⁴.

In a second step, the 47-mm diameter microscopic coil was placed near the right and left vascular pedicle. The 3D PCA acquisition with an encoding velocity of 10 cm/s (V10) and 30 cm/s (V30) allowed the positioning of two-dimensional cine phase-contrast MRI (2D CINE PC-MRI) quantitative sequences perpendicular to the arteries of the graft pedicle (Fig. 1). All technical parameters of the sequences used are presented in Table 1.

[Figure 1 here]

[Table 1 here]

Data management software

Two types of software were needed for angiographic, morphometric, and haemodynamic analysis.

During the acquisition, Advantage Windows software (General Electric Medical Systems, Milwaukee, WI, USA) allowed a reconstruction of the 3D PCA sequences in the three planes of space (axial, coronal, and sagittal) and in three dimensions, as well as a fusion of the V10 and V30 sequences. This made it possible to better identify the arterial component of the graft vascularization and to confirm the perpendicular positioning of the 2D CINE PC-MRI sequences with respect to the vessels of interest [Au?3].

In post-processing, the software 'Flow' developed by Balédent et al.¹¹ was used to extract morphometric data of sections and the average diameter, as well as haemodynamic measurements of average flow rates of the transplant arteries from the 2D CINE PC-MRI sequences. For accurate measurement of the vascular sections during the cardiac cycle, automated active contour segmentation (**Supplementary Material**, Video 1) based on the vector flow gradient algorithm¹⁵ was applied to the 2D CINE PC-MRI sequences.

Standardized clinical monitoring

This work formed part of the standardized monitoring of allotransplantations with collaboration between the teams of the Department of Maxillofacial Surgery of the University Hospital Centre of Amiens (France) and the Department of Transplantation, Nephrology, and Clinical Immunology of Edouard Herriot Hospital in Lyon (France). For each patient, the assessment included photographs, physical examination, and sequential biopsies performed on the sentinel skin flap or the facial allograft¹⁶.

Starting in 2014, a systematic analysis of the vascularization of the facial graft was performed by annual flow MRI acquisitions. The main objective of the study was to qualitatively and quantitatively evaluate the progression of the vascular capital of the facial transplant by flow MRI and to correlate these findings with the clinical and histological data.

Results

Patient 1

In 2005, at the age of 38 years, patient 1 underwent an allotransplantation of the nose and mouth triangle. The microsurgical anastomoses were performed on the right and left facial pedicles¹⁶. In 2013, the patient developed donor-specific antibodies and chronic humoral rejection⁴. The vascular assessment, performed by flow MRI in December 2014 and April 2015, showed a decreased bilateral signal recovery, predominantly right-sided (Fig. 2). This was confirmed by morphometric and

haemodynamic quantitative analyses, showing a significant reduction in arterial intraluminal diameter of 92.5% [Au?4] (2.73 mm vs. 0.75 mm) and reduction in average flow rate of 85.1% (26.8 ml/min vs. 4 ml/min) for the right facial artery, in comparison with reductions of 33.7% [Au?5] (2.77 mm vs. 2.6 mm) and 27.8% [Au?5] (52 ml/min vs. 37.5 ml/min) for the left facial artery. At the time of the second acquisition, erythematous macules had appeared on the right side of the graft (Table 2) [Au?3].

[Figure 2 here]

[Table 2 here]

At the 1-month post-acquisition assessment in May 2015, the patient had developed extensive right lower labial necrosis, which, for functional reasons, required partial resection of the transplant with anti-brachial [Au?6] free flap reconstruction. Pathological analysis of the resected graft showed multiple extensive thromboses with total intraluminal obstruction and perivascular neutrophil infiltration, characteristic of chronic vascular rejection⁴.

Patient 2

In 2009, at the age of 27 years, patient 2 underwent an allotransplantation of the lower third of the face (lip, chin, mandible). The microsurgical anastomoses were performed on the right and left facial pedicles. The course was complicated by the development of a large-cell Epstein–Barr virus-associated B-cell lymphoma followed by a hepatic post-transplant smooth muscle tumour, which made the adjustment of

immunosuppressive therapy particularly difficult¹⁷. Thus, after multiple episodes of rejection controlled by adjustment of the immunosuppressive treatment, the graft presented chronic scarring causing sclerosis, with no signs of chronic vascular rejection¹⁸.

Based on these conditions, a first flow MRI acquisition, performed in March 2015, demonstrated a permeable vascular capital with measured average flow rates for the right and left arterial pedicles of 66 ml/min and 20 ml/min, respectively, as well as intraluminal diameters corresponding to the standard of 1.785 mm (right) and 1.34 mm (left) (Fig. 3).

[Figure 3 here]

Since there was no regression of the hepatic tumours and no metastasis, the patient received a liver transplant in April 2017, after which he suffered from severe iterative [Au?7] sepsis episodes requiring intensive care and broad-spectrum antibiotic treatment. In June 2017 he presented with dry peri-oral ulcerations, raising suspicion of chronic vascular rejection. A second flow MRI acquisition revealed a significant reduction in the distal arterial signal, rendering it impossible to perform a quantitative acquisition (Table 2). Faced with the progression of the cutaneous lesions, partial resection of the graft was performed with an immediate anti-brachial [Au?6] flap reconstruction in July 2017.

Patient 3

In 2012, at the age of 52 years, patient 3 underwent allotransplantation of the tongue and lower and middle floors of the face after an initial resection of a voluminous high-flow haemorrhagic arteriovenous malformation, which was life-threatening despite numerous distal embolizations. The arterial anastomoses were performed in a termino-lateral manner between the external carotid grafts and the common carotids of the patient.

At 5 years post-transplantation, with no clinical evidence of chronic rejection or graft vasculopathy, follow-up by flow MRI initiated in 2015 displayed the absence of recurrence of the arteriovenous malformation, although a steady decrease in distal arterial signal recovery on 3D PCA angiographic sequences was observed. In addition, the latest acquisitions conducted in 2017 showed a total absence of signal in the previously existing right lingual artery (Fig. 4). Quantitative analyses confirmed this tendency. Over 2 years, a steady reduction in both morphometric values of intraluminal diameter of 21.6% and average flow rates of 60.2% was observed [Au'8] (Table 2).

[Figure 4 here]

Discussion

Evidence of the feasibility of flow MRI in vascular monitoring of facial VCA offers a new clinical and scientific perspective for future understanding and management of patients with VCA. In view of the increasing number of patients developing chronic

vascular rejection of VCA^{4,6,19}, the development of specific techniques for early diagnosis prior to the development of clinical symptoms seems timely.

Currently, while computed tomography angiography allows only an anatomical exploration, magnetic resonance angiography temporal sequencing, or CINE MRI, can estimate the speed of flow by displaying the duration of the contrast product washout, but without the possibility of a real quantitative analysis²⁰ [Au?3]. In addition, the injection of contrast agents is limited in transplant patients, due to the direct or indirect risks of acute renal failure and drug interactions.

Doppler ultrasound is a useful alternative for both anatomical and vascular haemodynamic exploration. Used by some teams in the vascular monitoring of the ulnar and radial axes of hand transplants⁵, its application in the head and neck area remains difficult because of the constraints related to the sinuosity of the external carotid artery, the infra-centimetric [Au?2] calibre of the external carotid branch arteries¹⁴, and the numerous bony interpositions. This decreases the precision of quantitative measurements and their reproducibility.

Based on the principle of spin-phase shift⁷⁻⁹, flow MRI offers the advantage of being an accurate and standardized examination¹³. By adapting the acquisition protocol to the problem of vascular monitoring of facial VCA, focusing mainly on the vascular pedicles, the examination time could be reduced to an average duration of 28 minutes. Moreover, it offers a qualitative analysis by 3D PCA sequences with the possibility of a quantitative measurement of the artery of interest through kinematic 2D CINE PC-MRI sequences.

Having completed regular clinical and pathological monitoring, a progressive reduction in both intravascular diameter and average flow rate could be observed in patients 1 and 3, before any definitive sequelae attributable to vascular rejection,

through the examination of the flow MRI acquisitions (these patients underwent at least two acquisitions by flow MRI). Although the progressive and consistent characteristics of chronic vascular rejection associated with intimal proliferation of smooth muscle cells is known²¹, the present study results are novel in demonstrating a reduction in graft perfusion flow prior to acute vascular stenosis.

A decrease in the right and left facial artery flow rates of 85.2% and 27.8% [Au?9] were observed in patient 1, and of 59.4% and 60.1% [Au?10] in patient 3, versus an intraluminal diameter reduction of 72.44% [Au?11] (right) and 18.5% [Au?11] (left) in patient 1 and of 6.75% [Au?12] (right) and 16.4% [Au?12] (left) in patient 3 ($P < 0.05$, by Fisher's test). These results were compared to the qualitative analysis using 3D PCA sequences of the graft vascular capital, which in all three patients revealed a complete absence of vascular graft autonomy. Thus, several years after transplantation, no peripheral neovascularization was detectable. The graft appeared haemodynamically isolated from the recipient site, making the transplant solely dependent on its primary arterial pedicle and therefore vulnerable to any episode of thrombosis or hypovolemia. During the episodes of severe sepsis in patient 2, reactive cyanosis of the transplant that improved spontaneously during the recovery of haemodynamic findings was observed [Au?3]. Therefore, there appears to be a fragility of the graft towards mechanical, cytotoxic, immune, or thermal factors, leading to intimal hyperplasia (?) [Au?13] and pathological vascular remodeling²²⁻²⁴. This suggests a complete absence of peripheral vascular compliance, the graft behaving indefinitely as a separate and non-integrated entity.

By reporting these preliminary haemodynamic results on the progression of the vascular capital of facial VCA, this study highlights flow MRI as a technique of choice in the assessment of autoimmune vasculopathies. Nonetheless, the results

obtained in patient 3 are not in line with this interpretation of the anatomical–haemodynamic dissociation (decrease in the average flow by 60.2% over 3 years, versus a decrease by 11.6% in the arterial intraluminal diameter [Au?14]). Acting independently of rejection with clinical lesions, this dissociation was physiological and secondary to a haemodynamic equilibration of the graft at 5 years, leading us to suspect subclinical vascular rejection. Between 2014 and 2016, a significant change in the signal in the right lingual artery had already been observed, with a flow rate that became impossible to measure between 2015 and 2016, indicating an active subclinical vascular process [Au?15].

Apart from patient 3, no histological evidence was found in biopsies performed on the sentinel skin flap. In this context, it is legitimate to assess the sensitivity of flow MRI with respect to histological examination concerning the characterization and identification of vascular rejection lesions, all the more so because deep biopsies induce significant scarring sequelae in the long-term follow-up. The observation in 2017 (patient 3) made it possible to identify a vascular compliance of the graft arteries, despite the obliterating involvement of the right lingual artery. These observations suggest that the facial pedicle is the last artery affected, but its obliteration causes the first clinical signs of chronic rejection. The absence of preceding lesions on the sentinel flap may be explained by its terminal vascularization, whose calibre is close to that of the facial artery (approximately 2 mm in diameter)^{25,26}. Therefore, an ideal model for monitoring the graft would be to perform iterative [Au?77] biopsies where, by analogy, the results obtained are transposable to those observable if the biopsies were performed directly on the transplant.

Our experience appears to confirm this synchronous development between the grafts and sentinel skin flaps. In patients 1 and 2, the appearance of signs of rejection occurred within 2 months. However, this synchronicity does not predict the development of humoral rejection. The prospect of identifying a subclinical threshold of vascular fragility of the graft could allow the transplant team to adapt the immunosuppressive treatment as soon as possible while having the time to design a replacement or re-transplantation strategy, avoiding an emergency surgery related to extensive graft necrosis.

In conclusion, while chronic vascular rejection is one of the major complications of long-term VCA, evidence of the feasibility of accurate, objective, and non-invasive vascular flow MRI monitoring for facial VCA opens new perspectives for future understanding and management of allotransplant patients. The identification of a critical vascular threshold characteristic of each VCA could make it possible to establish therapeutic strategies to slow the progression of chronic rejection and limit the risk of emergency re-transplantation. Nevertheless, tracking facial VCAs is only one of many possible indications of flow MRI. Indeed, this technique opens new perspectives to document and better understand the haemodynamic phenomena secondary to microanastomosis tissue transfer.

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Competing interests

No conflict of interest to declare.

Ethical approval

This study was approved by the local ethics committee (No. 2013-A00319-36). It was registered in clinicaltrials.gov (NCT 02829190) and was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Patient consent

Written and signed consent was obtained for each patient.

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Figure captions

Fig. 1. (a) 3D PCA acquisition (encoding velocity 30 cm/s) of the external carotid vasculature with the 32-element head coil (insert a) used in the qualitative analysis of the graft vascular capital and for locating the graft arterial pedicle in order to position the 47-mm microscopic coil (insert in d) near the vessel of interest. 3D PCA acquisition of the artery pedicle with the 47-mm diameter microscopic coil with an encoding velocity of (b) 30 cm/s (V30) and (c) 10 cm/s (V10). (d) Fusion of the V10 and V30 sequences was used to position the 2D CINE PC-MRI sequence perpendicular to the artery of interest and extract the time variation of (e) mean velocity, (f) vessel cross-section, and (g) flow rate over the cardiac cycle.

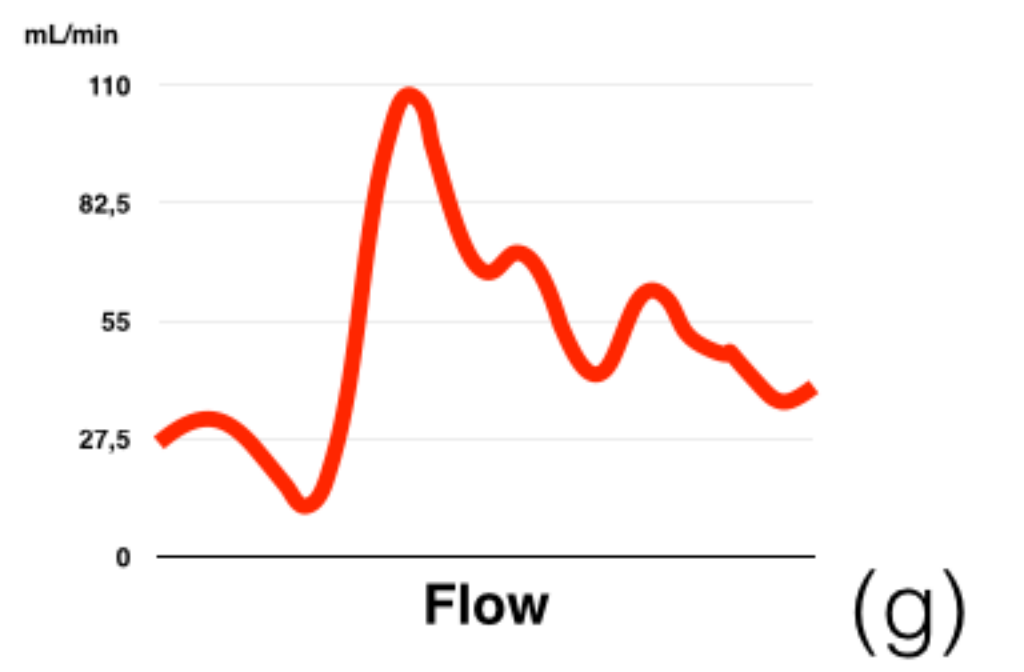
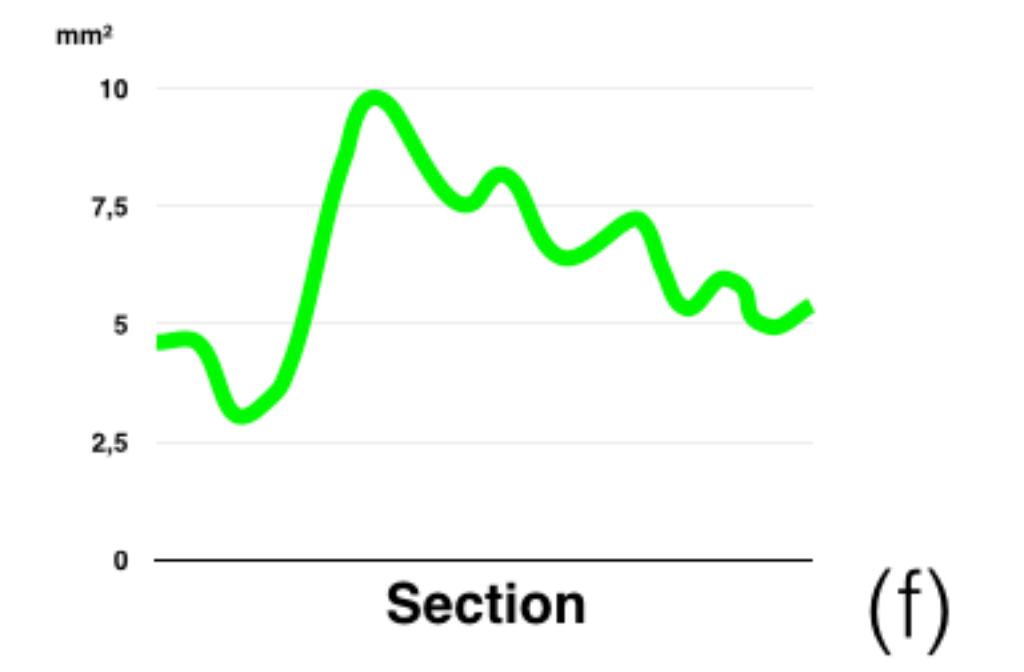
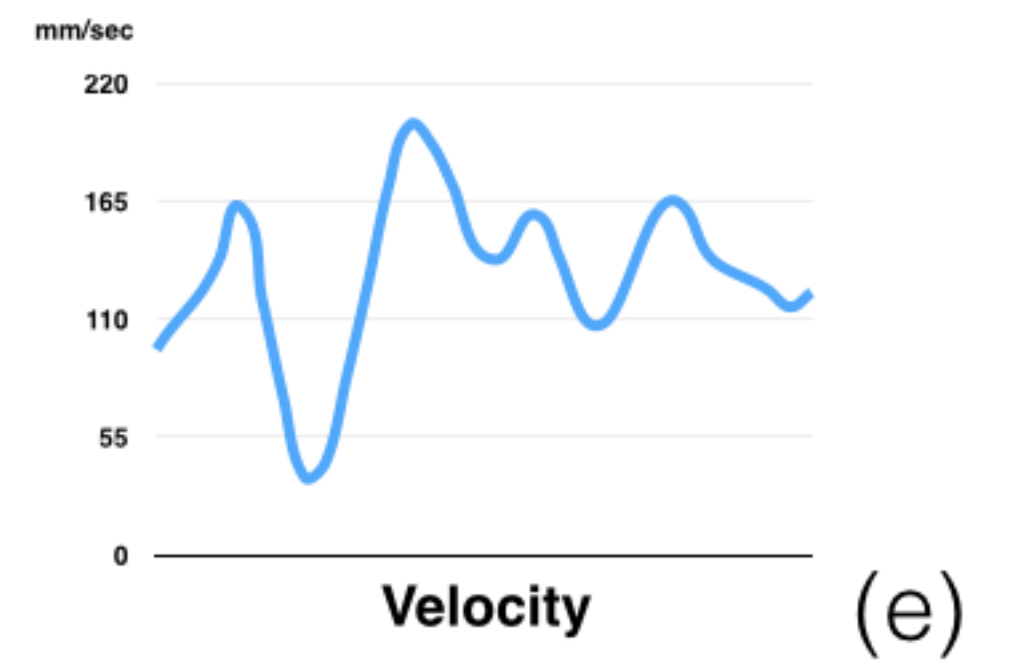
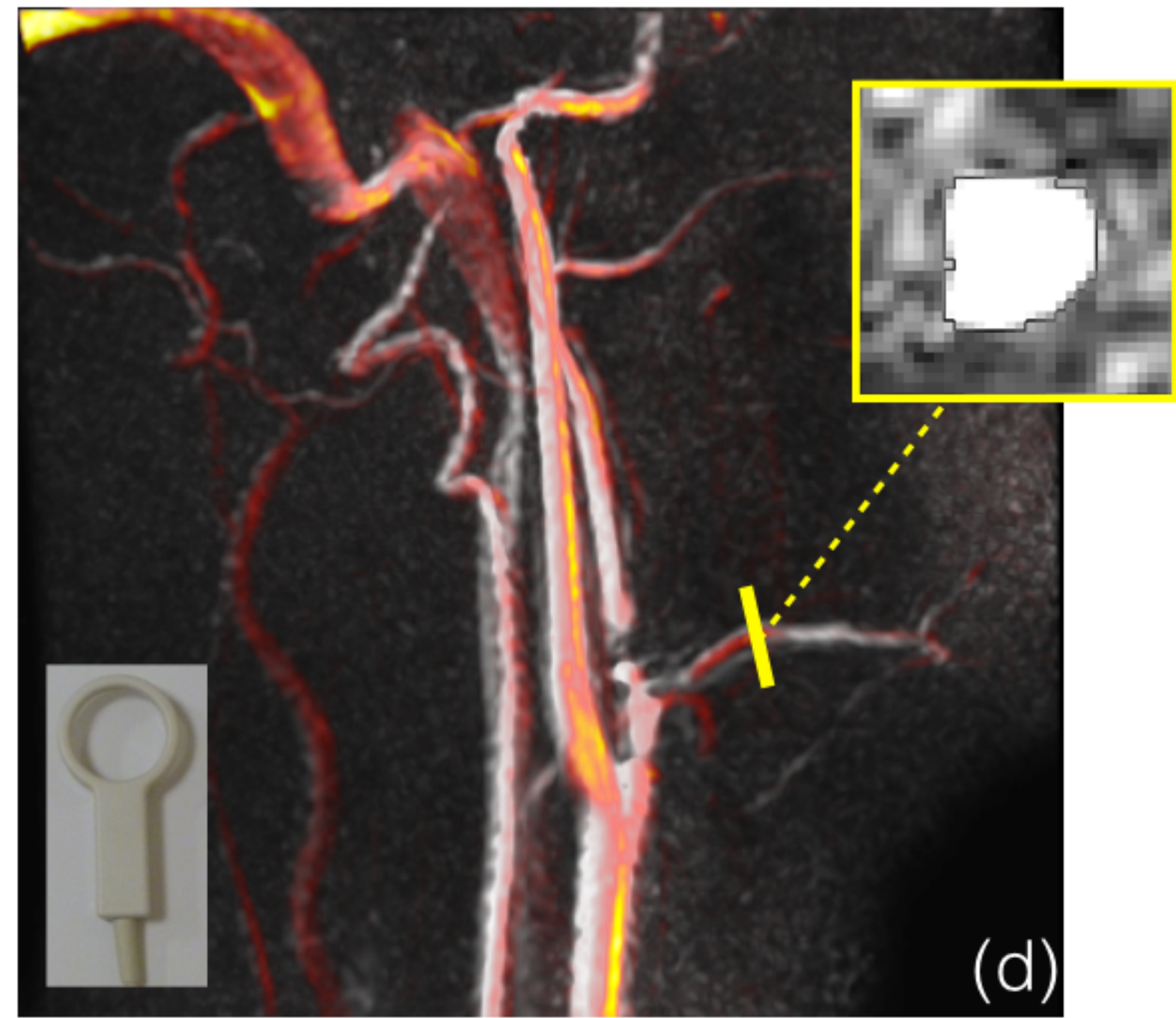
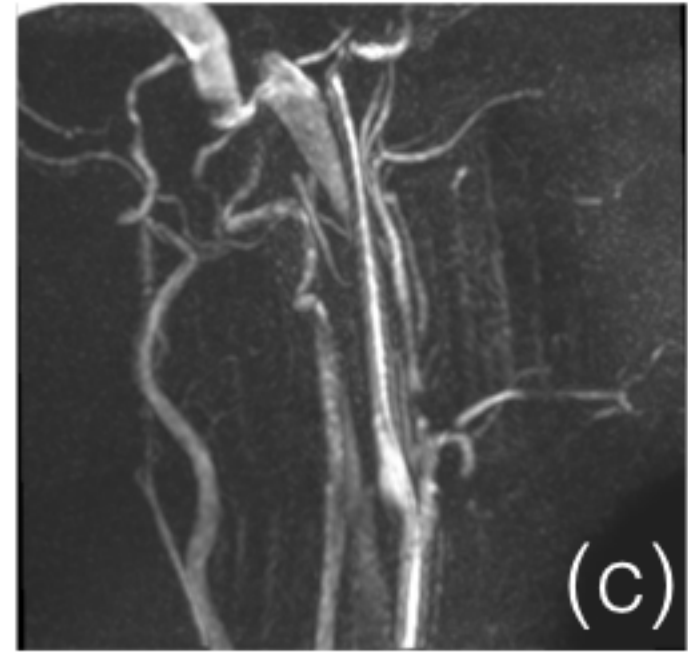
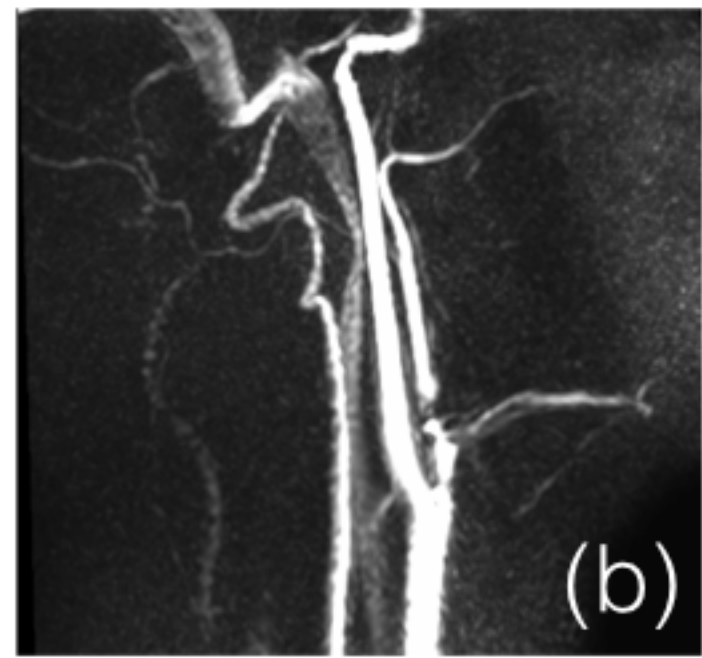
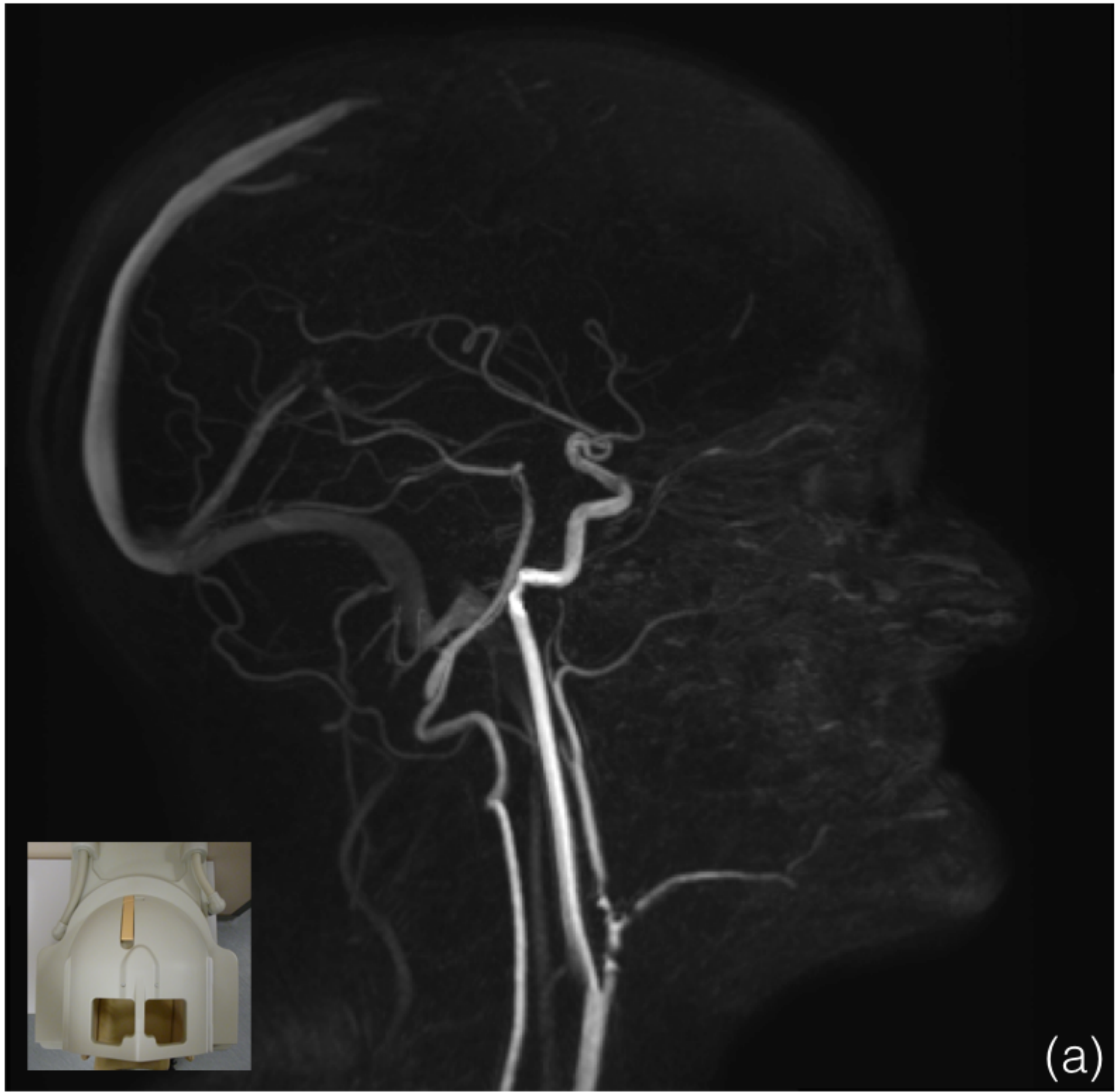
Fig. 2. Patient 1: Comparative analysis of the vascular capital of the graft using 3D PCA sequences (encoding velocity 30 cm/s) obtained in December 2014 and April 2015. In these acquisitions, a decrease and obliteration (white circle) of MRI signal in the left and right facial arteries (FA) of the graft can be seen, along with the appearance of facial erythematous macules. [Au?3]

Fig. 3. Patient 2: Comparative analysis of the vascular capital of the graft using 3D PCA sequences (encoding velocity 30 cm/s) obtained in March 2015 and June 2017. In the images from June 2017, complete obliteration of the MRI signal of both facial arteries (FA) can be observed (white circle), along with the appearance of dry lesions indicating chronic vascular rejection. [Au?3]

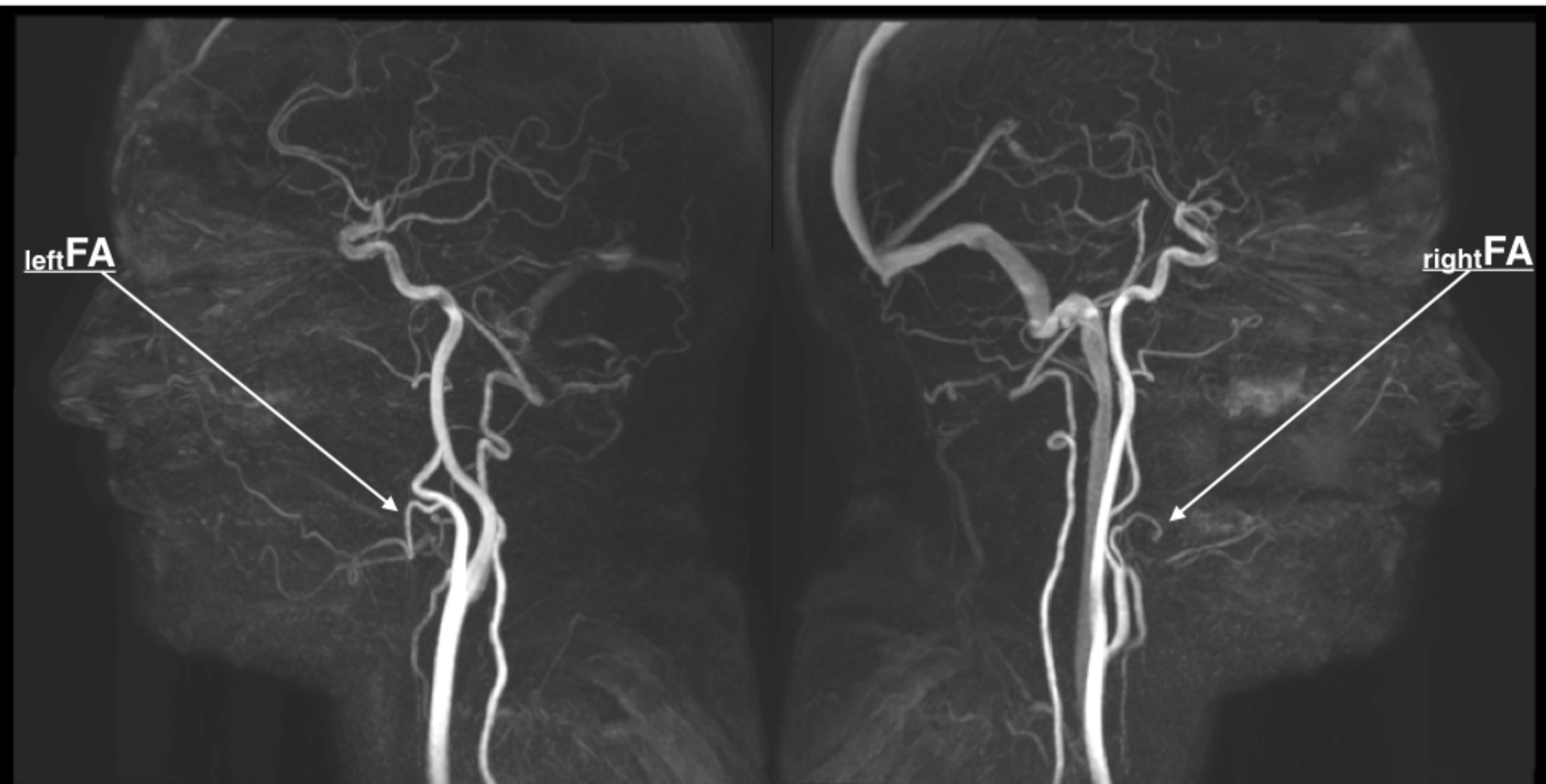
Fig. 4. Patient 3: Comparative analysis of the vascular capital of the graft using 3D PCA sequences obtained between February 2015 and December 2017. At 5 years post transplantation, with no clinical evidence of chronic skin rejection or graft vasculopathy, qualitative analysis of the arterial vascular capital showed a constant decrease in the MRI signal of the graft arteries between 2016 and 2017; a qualitative and quantitative analysis of the left facial artery (leftFA) was only possible with the 47-mm diameter microscopic coil acquisitions (insert). An obliteration of the MRI signal in the right lingual artery (rightLA) was observed between 2016 and 2017 (insert), rendering haemodynamic measurements impossible. [Au?3]

Supplementary Material

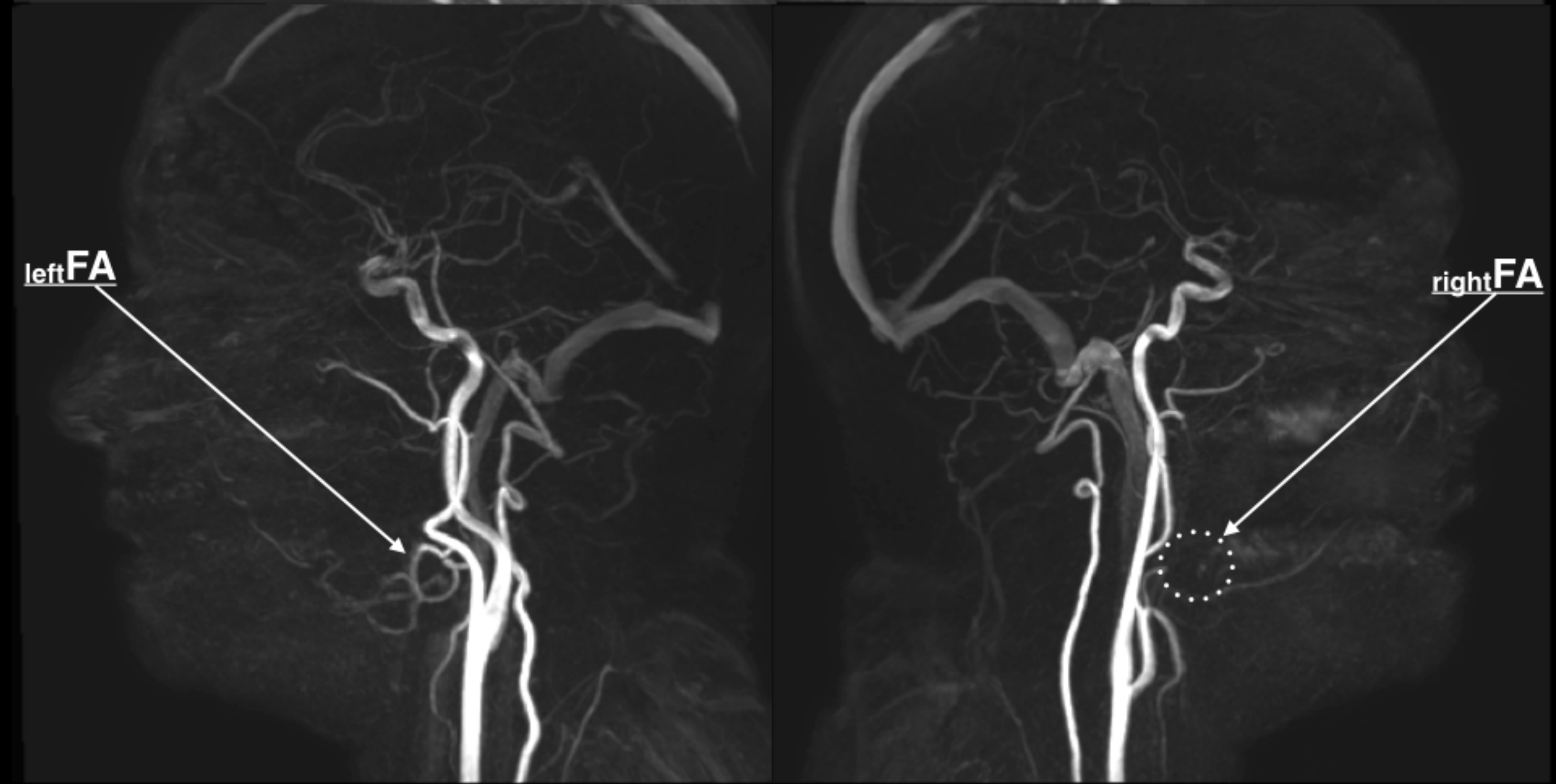
Video 1. Example of an automated active contour segmentation of a facial artery: this segmentation technique tracks both movements and diameter variations during the cardiac cycle, providing accurate qualitative and quantitative measurements with minimal information loss.



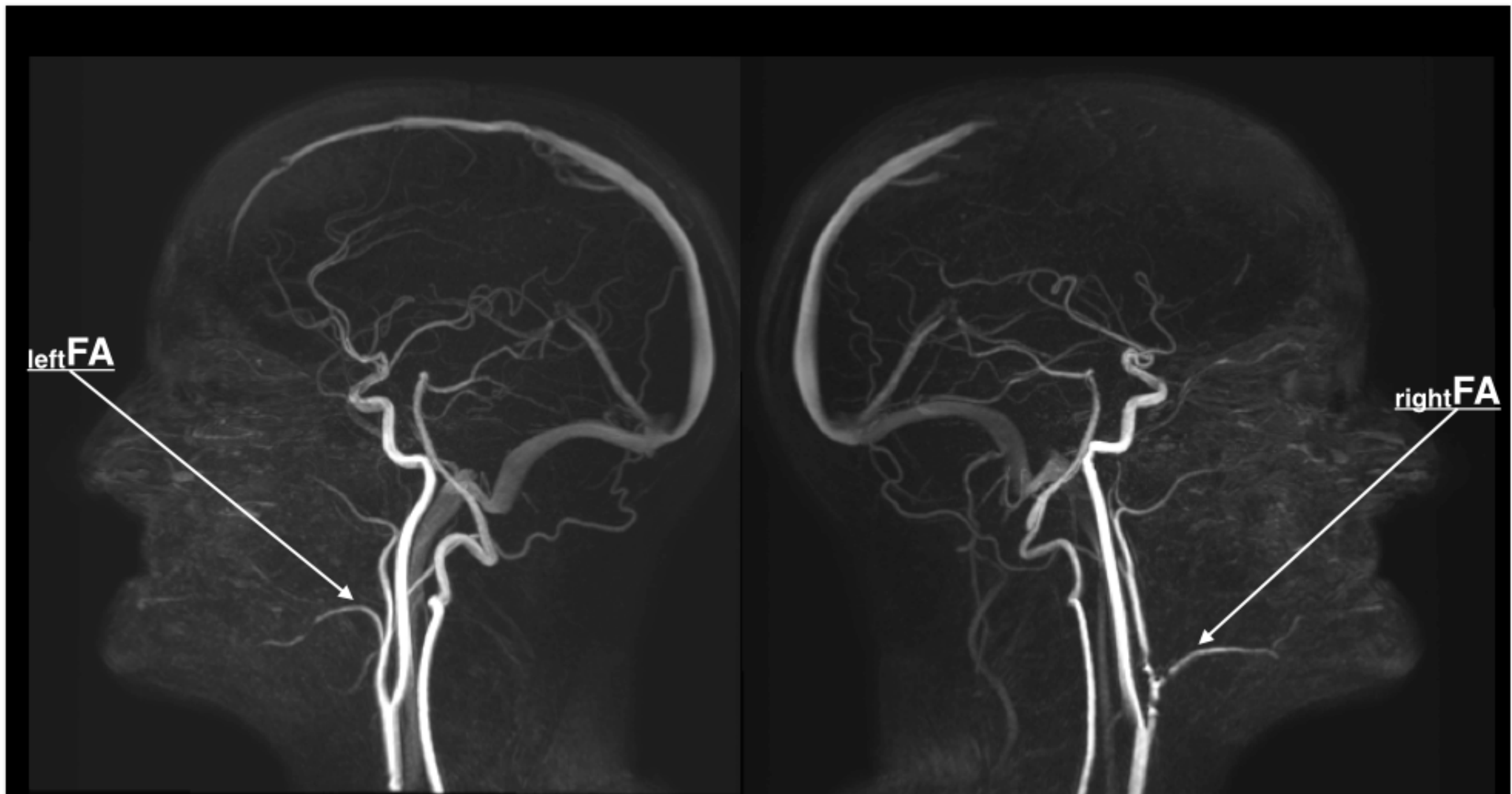
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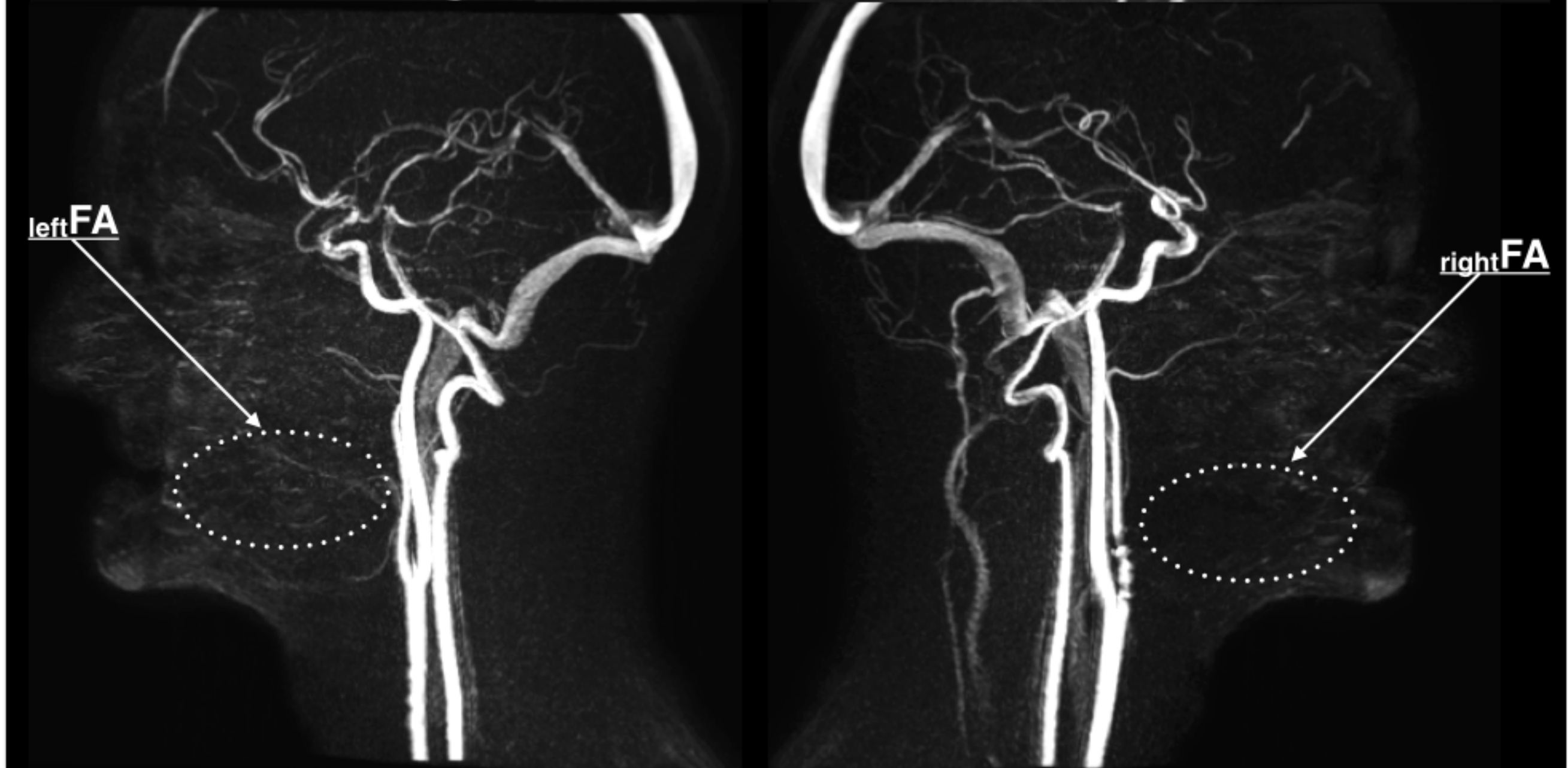
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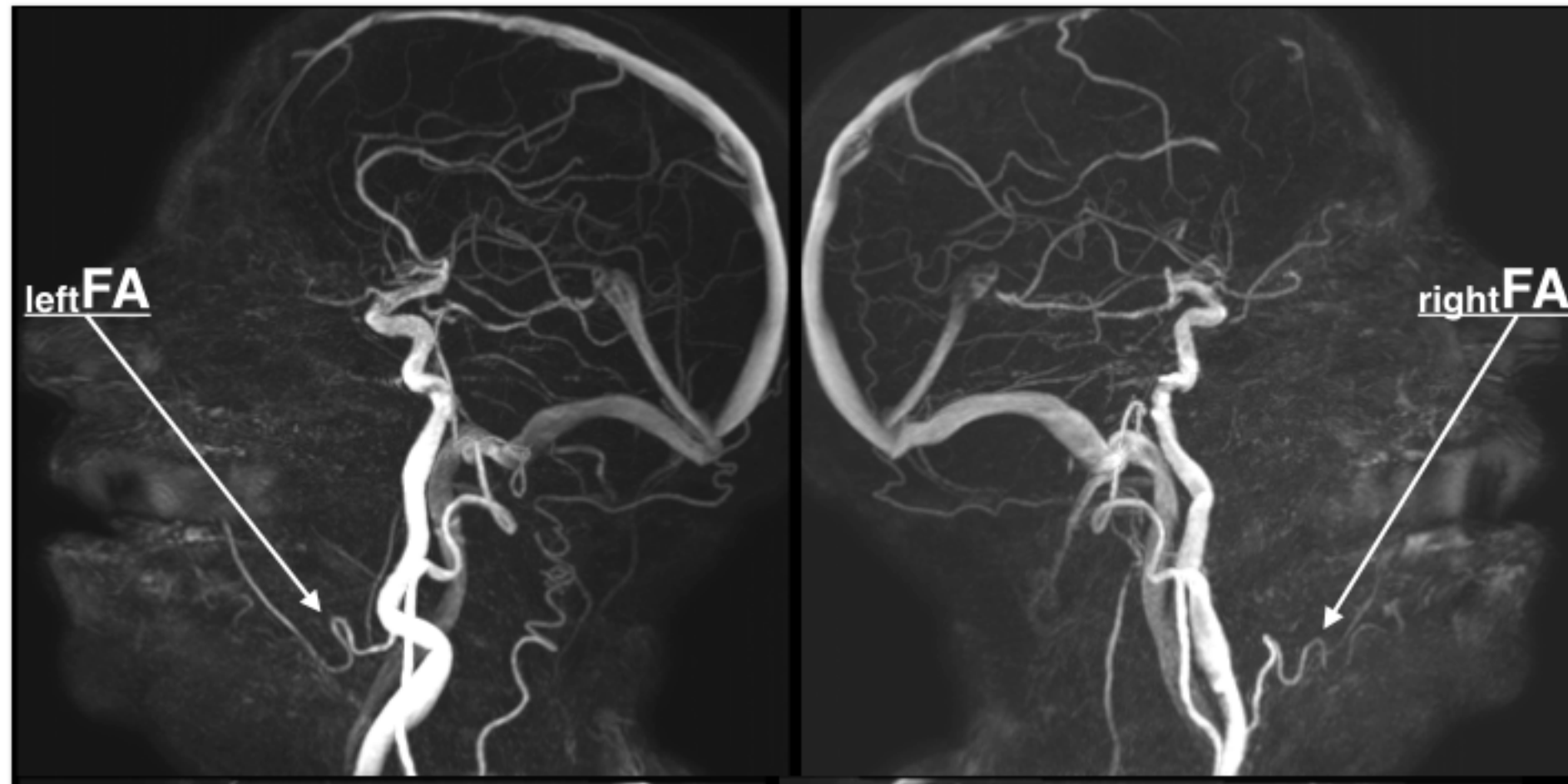
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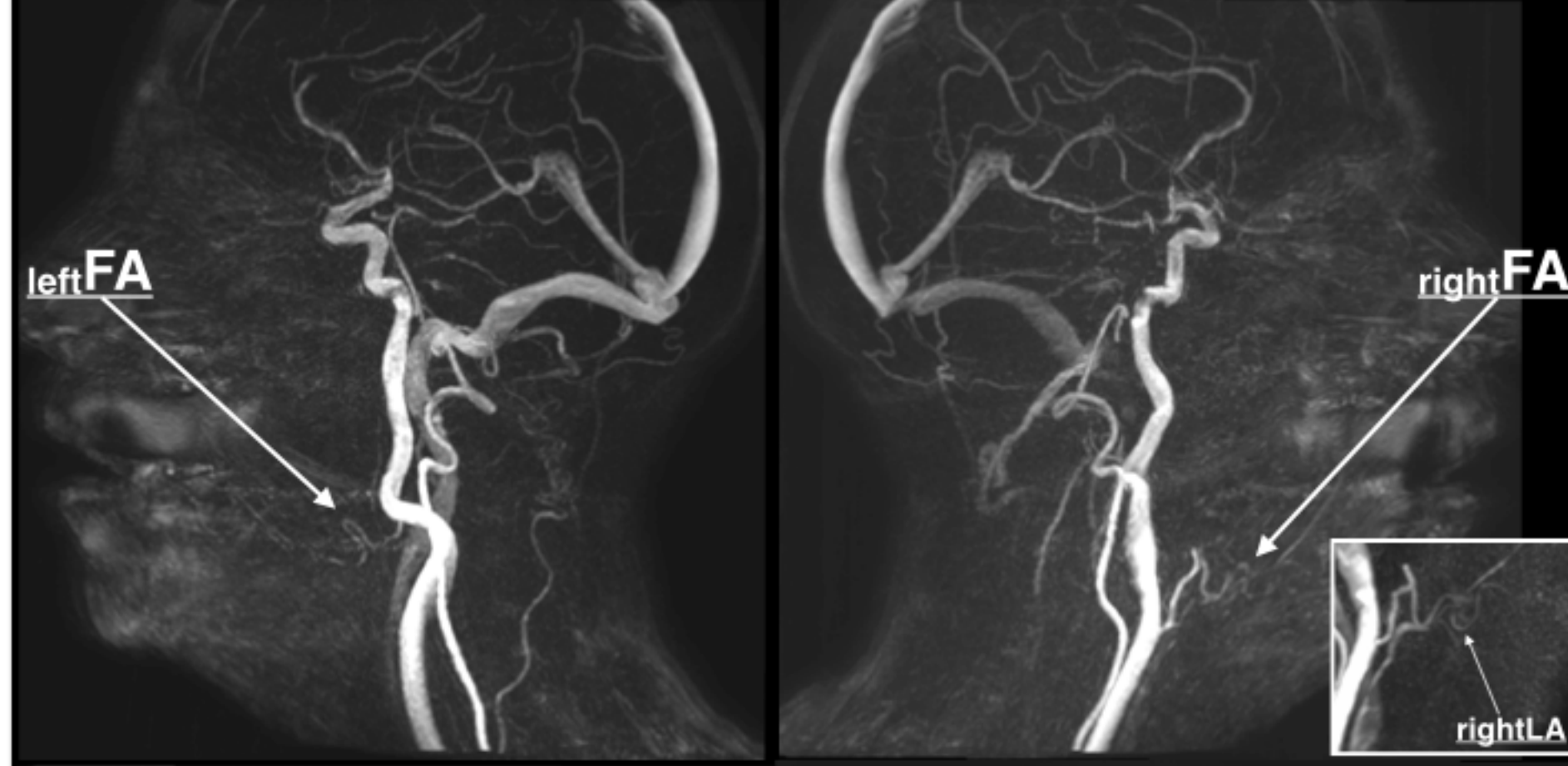
June 2017



February 2015



February 2016



December 2017

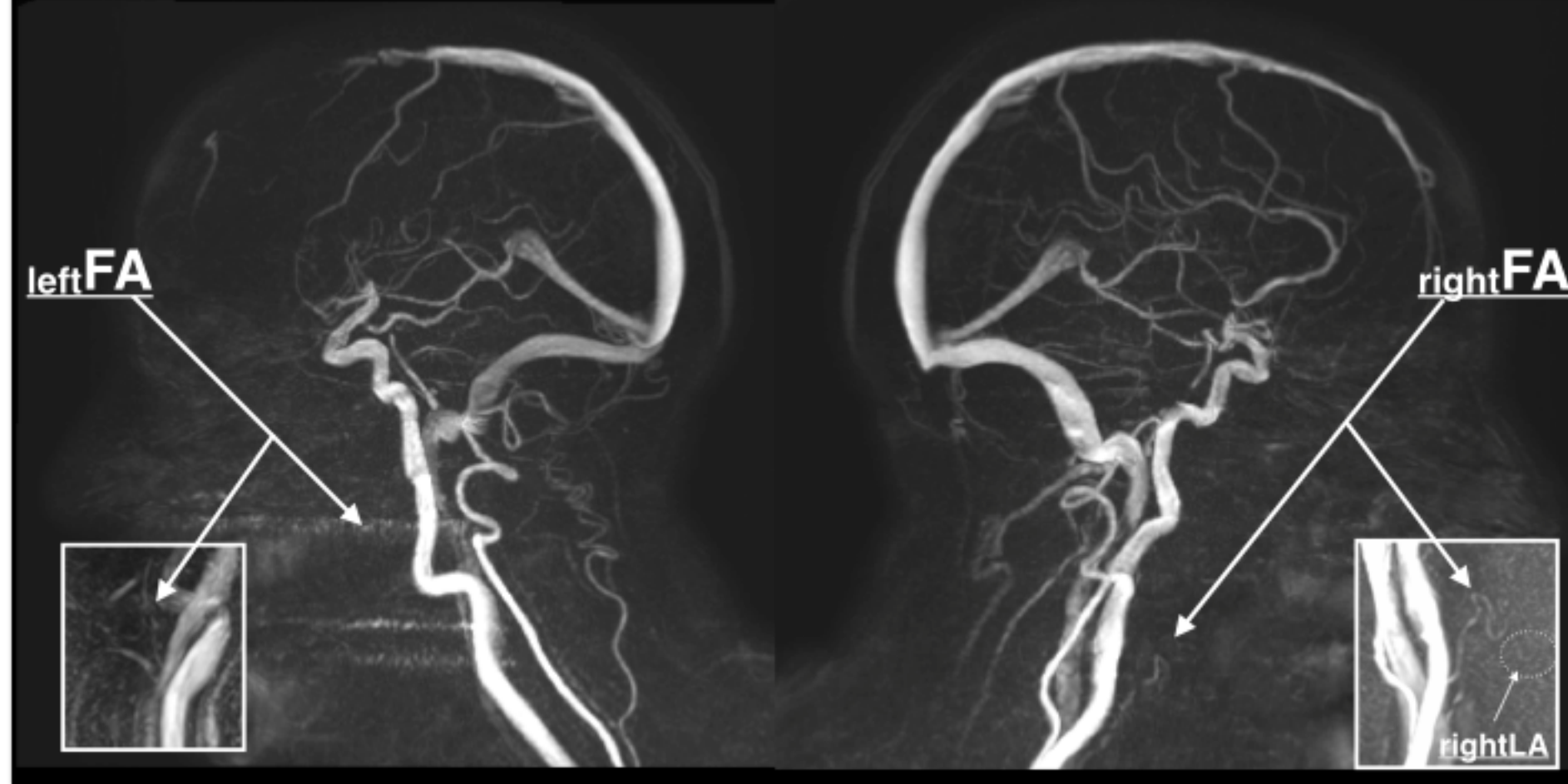


Table 1. 3D PCA and 2D CINE PC-MRI sequence parameters.

	Sequence		
	3D PCA	3D PCA	2D CINE PC-MRI
	32-head coil	47-mm coil	47-mm coil
TE/TR (ms)	3/5	3/5	13/8
Spatial resolution (mm ³)	0.5 × 0.5 × 1	0.6 × 0.6 × 1.2	0.15 × 0.15 × 2
FOV (mm ²)	240 × 240	140 × 140	50 × 50
Encoding velocities (cm/s)	30	30 and 10	between 25 and 45
Number of frames per cardiac cycle	1	1	16
Average duration	6 min 20 s	4 min 47 s	2 min 23 s

2D CINE PC-MRI, two-dimensional cine phase-contrast magnetic resonance imaging; 3D PCA, three-dimensional phase-contrast angiography;

FOV, field of view; TE, echo time; TR, repetition time.

Table 2. Morphometric and haemodynamic quantitative analysis of the pedicle arteries of the graft in patients 1, 2 and 3.

	Patient 1			Patient 2			Patient 3		
Year of transplantation	2004			2009			2012		
Initial loss of substance	Nose and mouth triangle			Lip, chin, mandible			Tongue, lower and middle face floors (mandible, maxilla)		
	Date of acquisition	Data	Change	Date of acquisition	Data	Change	Date of acquisition	Data	Change
Right facial artery									
Average flow (ml/min)	December 2014	26.8	-85.1%	March 2015	66	-	February 2015	25	-59.4%
	April 2015	4		June 2017	MI		February 2016	18.24	
							December 2017	10.14	
Intraluminal diameter (mm)	December 2014	2.73	-72.5%	March 2015	1.785	-	February 2015	1.3	-7.7%
	April 2015	0.75		June 2017	MI		February 2016	1.2	
							December 2017	1.2	
Left facial artery									
Average flow (ml/min)	December 2014	52	-27.9%	March 2015	20	-	February 2015	48.5	-60.8%
	April 2015	37.5		June 2017	MI		February 2016	38.2	

							December 2017	19	
Intraluminal diameter (mm)	December 2014	2.77	-6.2%	March 2015	1.34	-	February 2015	1.79	-16.2%
	April 2015	2.6	[Au?17]	June 2017	MI		February 2016	1.97	
								[Au?18]	
							December 2017	1.5	
Right lingual artery									
Average flow (ml/min)							February 2015	14.4	-
							February 2016	13.27	
							December 2017	MI	
Intraluminal diameter (mm)							February 2015	1.33	-
							February 2016	1.23	
							December 2017	MI	

MI, measurement impossible.