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EVALUATION OF PRO RE NATA AND TREAT AND EXTEND ANTI-VEGF PROTOCOLS IN SORSBY FUNDUS DYSTROPHY

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Abstract:	Abstract Purpose: To describe long term outcomes with intravitreal Bevacizumab for choroidal neovascularization (CNV) secondary to Sorsby Fundus Dystrophy (SFD). Materials/Methods: Observational case series. Results: Two sisters of the same family formally diagnosed with SFD were followed-up for 12 years. The elder sister (S1) presented with significant decline in vision due to CNV in her right eye (OD). She developed CNV 3 years later in her left eye (OS). She was treated with Bevacizumab intravitreal injections (IVIs) on a Pro Re Nata (PRN) basis until April 2015, when a Treat and Extend (T&E) approach was adopted. Best corrected visual acuities (BCVA) at the time of switch to T&E were 1.09 OD and 0.85 LogMar OS. BCVAs at the last follow-up were LogMar 1.1 OD and 0.82 OS. Her younger sister (S2) presented with BCVAs of LogMar 0.1 OD and 0.0 OS. She developed CNV 5 years in both eyes. OS developed CNV 18 months after her right eye. She received Bevacizumab on a PRN basis until April 2015 when a switch to a T&E was performed. BCVA in the left eye at the switch to T&E was 0.34 LogMar. At the last follow-up, BCVAs were LogMar 1.2 OD and 0.29 OS. Conclusions: Bevacizumab is an effective therapy for CNV secondary to SFD. A T&E protocol appears more effective compared to PRN protocol in minimising recurrence of CNV with potential secondary scar formation or atrophy.
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Full Title

EVALUATION OF PRO RE NATA (PRN) AND TREAT AND EXTEND ANTI-VEGF PROTOCOLS IN SORSBY FUNDUS DYSTROPHY

Short Title

Choroidal Neovascular Membrane treatment outcomes in Sorsby Fundus Dystrophy

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Abstract

Purpose: To describe long term outcomes with intravitreal Bevacizumab for choroidal neovascularization (CNV) secondary to Sorsby Fundus Dystrophy (SFD).

Materials/Methods: Observational case series.

Results: Two sisters of the same family formally diagnosed with SFD were followed-up for 12 years.

The elder sister (S1) presented with significant decline in vision due to CNV in her right eye (OD). She developed CNV 3 years later in her left eye (OS). She was treated with Bevacizumab intravitreal injections (IVIs) on a Pro Re Nata (PRN) basis until April 2015, when a Treat and Extend (T&E) approach was adopted. Best corrected visual acuities (BCVA) at the time of switch to T&E were 1.09 OD and 0.85 LogMar OS. BCVAs at the last follow-up were LogMar 1.1 OD and 0.82 OS.

Her younger sister (S2) presented with BCVAs of LogMar 0.1 OD and 0.0 OS. She developed CNV 5 years later in both eyes. OS developed CNV 18

months after her right eye. She received Bevacizumab on a PRN basis until April 2015 when a switch to a T&E was performed. BCVA in the left eye at the switch to T&E was 0.34 LogMar. At the last follow-up, BCVAs were LogMar 1.2 OD and 0.29 OS.

Conclusions: Bevacizumab is an effective therapy for CNV secondary to SFD. A T&E protocol appears more effective compared to PRN protocol in minimising recurrence of CNV with potential secondary scar formation or atrophy.

Introduction

Sorsby Fundus Dystrophy (SFD) is an autosomal dominant (AD) retinal degeneration due to mutation in the gene encoding Tissue Inhibitor Metalloproteinase-3 (TIMP-3) (1, 2). TIMP-3 is one of four members of a family of proteins that were originally classified according to their ability to inhibit matrix metalloproteinases (MMP). This protein is a potent inhibitor of angiogenesis. Inhibition of angiogenesis is achieved by blocking the binding of Vascular Endothelial Growth Factor (VEGF) to VEGF receptor-2 (3).

Unlike TIMP-1, TIMP-2 and TIMP-4, which are all water soluble proteins, TIMP-3 is not water soluble and is expressed in the eye localized as part of Bruch's membrane (4). It is unclear whether protein variants in TIMP-3 retain the ability to inhibit anti-VEGF receptor signalling (5). Choroidal neovascularization (CNV) in SFD usually causes central visual loss between

the third and fourth decade of life (1, 6). In the United Kingdom, most patients with SFD carry the Ser204Cys mutation in exon 5 of the TIMP-3 gene.

Ophthalmic examination of patients with SFD shows deposition of drusen at the posterior pole during the initial stages of the disease (7). Later, more pronounced macular involvement is observed with the formation of secondary CNV or diffuse macular atrophy. The peripheral retina is not spared in SFD; peripheral retinal atrophy can result in loss of ambulatory vision in the seventh decade of life (7).

Up until the discovery of anti-VEGF therapy, CNV was either not treated or was treated with poor outcomes after thermal or photodynamic laser (8). Bevacizumab (Avastin, Genentech, South San Francisco, CA) is a humanized recombinant antibody that binds all isoforms of VEGF (9). It is used off-label for the treatment of CNV secondary to a wide range of ocular diseases. Ranibizumab (Lucentis) is a smaller monoclonal antibody fragment that also inhibits VEGF. Lucentis is, however, FDA approved for the treatment of neovascular age-related macular degeneration (nAMD). Both anti-VEGF factors have been recently used for the treatment of CNV secondary to SFD (9-12).

Here, we describe multimodal imaging findings in two sisters, who were both diagnosed with SFD induced CNV and the response to Pro Re Nata (PRN) and Treat and Extend (T&E) treatment protocols over a 12 year period. At the moment, these are the only patients with CNV secondary to SFD receiving treatment in our department with a long follow up period (12 years) that would

permit a comparison between different treatment protocols of anti-VEGF delivery.

Materials and Methods

A retrospective case series at the Eye Unit of University Hospital Southampton NHS Foundation Trust was performed. The two sisters were followed-up over a period of 12 years. Signed consent for therapy with Bevacizumab was obtained from both sisters. Visual acuity assessment and complete ophthalmic examination including multimodal imaging were performed. Multimodal imaging included colour fundus photography, Optical Coherence Tomograms (OCT), Optical Coherence Tomography Angiography (OCT-A), Fundus Autofluorescence (FAF) and Fundus Fluorescein Angiography (FFA). Fundus photography and all the multimodal imaging investigations mentioned above were obtained with a confocal scanning laser ophthalmoscope (Spectralis HRA-OCT; Spectralis HRA-OCT-A; Spectralis HRA FFA; Heidelberg Eye Explorer, Version 1.9.17.0, Heidelberg Engineering, Heidelberg, Germany).

Mean age at development of CNV in the first eye, time between CNV development in first and second eyes, change in visual acuity with Bevacizumab treatment, and change in central retinal thickness (CRT) are described. With multimodal imaging, the macular anatomical changes were assessed over time.

Results

The patients' family tree demonstrated that their father, paternal aunt, paternal grandmother and paternal great grandfather suffered from the disease. Based on the family tree, the disease could be traced back to the 18th century. Both sisters were tested and found to carry the same mutation in the exon 5 of the TIMP-3 gene (Ser204Cys).

The elder sister (S1) presented initially in September 2006 at a different NHS Eye unit at an age of 34 years with significant decline in her visual acuity in her right eye (6/60 Snellen, 1.0 LogMar) and signs suggestive of right CNV. In 2006, there was a lengthy application process for anti-VEGF treatment to be provided within the NHS. Therefore, S1 elected to go privately for her injections. She then received privately two injections of Bevacizumab at a 4week interval. After the two intravitreal injections of Bevacizumab, her right eye visual acuity improved to 6/6 OD. The patient was then referred to the inherited diseases clinic at the Southampton Eye Unit, University Hospital Southampton. At her 1st assessment in January 2007, her left eye did not exhibit any sign of CNV secondary to SFD and the right eye CNV was inactive. In February 2007, the right eye CNV recurred and the patient received her first NHS Bevacizumab IVI (3rd Bevacizumab IVI). S1 received two more Bevacizumab IVIs in her right eye (a total of five Bevacizumab IVIs in the right eye) on a PRN basis until a disciform scar was formed (July 2007). Anti-VEGF IVIs treatment was stopped due to the formation of end-stage

disease. In January 2009, the patient noted left eye metamorphopsia (BCVA 6/4 [-0.1 LogMar]). She was found to have an active extra-foveal CNV with subretinal fluid. One session of photodynamic therapy (PDT) was carried out as an initial treatment instead of intravitreal Bevacizumab treatment with no significant response. A month later, she was treated with Bevacizumab IVIs on a PRN basis from the time of CNV presentation until April 2015. The time between the left eye PDT and the initiation of intravitreal Bevacizumab treatment was a month.

Since then, treatment with Bevacizumab has been continued on a T&E protocol. BCVAs at the time of switch to T&E were 1.09 and 0.85 LogMar in the right and left eye respectively. Since January 2009, this patient has received a total of 79 Bevacizumab IVIs in the left eye. In her last follow-up, BCVAs of 1.1 and 0.82 LogMar in the right and left eyes respectively were documented. Until April 2015, the elder sister's left eye received 58 Bevacizumab IVIs on a PRN basis. From April 2015 until now, her left eye has received 21 injections on a T&E protocol. Tables 1and 2 summarize the genetic, phenotype and clinical features of both eyes for both sisters.

The younger sister (S2) presented at Southampton Eye Unit in May 2007 for close monitoring after her elder sister (S1) was diagnosed with SFD. BCVAs at presentation were -0.1 and 0.0 LogMar of the right and left eyes respectively. The patient was asymptomatic and she remained so until March 2012 (was 36 years old then), when CNV was observed on dilated fundus exam of the right eye and on multimodal imaging. At that time, S2 was 8-

weeks pregnant. Her left eye at that stage showed only deposition of drusen at the posterior pole. As the patient was 8-weeks pregnant with her second child, no anti-VEGF IVIs were given to avoid potential teratogenicity. The pregnancy did not proceed and she received her 1st Bevacizumab IVI in July 2012 (4-month delay in initiation of treatment due to the fact that she was previously pregnant). 18 months after right eye (OD) CNV diagnosis, her left eye (OS) was diagnosed for the 1st time with active CNV secondary to SFD. The right eye received a total of 24 Bevacizumab IVIs on a PRN basis, the macula showed features suggestive of scar tissue formation and secondary atrophy hence anti-VEGF treatment was stopped with a documented VA of 1.07 LogMar. The left eye received in total 6 Bevacizumab IVIs on a PRN basis until April 2015. Since then, an attempt was made to continue treatment with the same anti-VEGF but on a Treat and Extend protocol. BCVA at the time of the switch to Treat-and-Extend was 0.34 LogMar. If the inter-injection interval was extended beyond 6 weeks, the CNV activity recurred. For that reason, she has continued with an inter-injection interval at Q4W (monthly). Her right eye received 24 Bevacizumab injections on a PRN basis in total until end-stage disease developed, whereas the left eye had received 36 Bevacizumab injections on fixed 4 weekly intervals. In total, the younger sister's left eye received 42 Bevacizumab IVIs. No PDT was offered. BCVAs of 1.2 and 0.29 LogMar were documented at the last follow up visit of the right and left eyes respectively. No significant complications post injections were diagnosed in either eye in both sisters. Figure 1 shows the BCVA over time in both sisters.

Multimodal imaging with serial OCT scans demonstrated progressive scarring and fibrosis in both eyes despite the treatment with Bevacizumab (Figure 2). FFA revealed absence of leakage in early stages but late staining due to extensive fibrosis and scarring in the right eye of both S1 and S2 (Figure 3). S1's FFA of the left eye revealed a focal point of leakage at the early stages and late staining at the later stages (Figure 4A). S2's FFA revealed a focal point of leakage increasing in size and intensity (Figure 4B) on PRN treatment. Interestingly after switch to T&E protocol with IVI Bevacizumab, S2's OCT-A showed CNV despite no evidence of sub-retinal fluid on OCT scans (Figure 5). S1's multicolour imaging revealed extensive macular changes due to the treated CNV (Figure 6). Similar findings were detected in S2's multicolour imaging (data not shown).

Discussion

Treatment of CNV secondary to SFD remains quite challenging. Sivaprasad et al (8) described a limited case series, where eyes with CNV secondary to SFD received argon laser photocoagulation, PDT alone or in combination with intravitreal triamcinolone or intravitreal bevacizumab (8). Holz and colleagues described a case series of ten eyes with aggressive recurrence of an extrafoveal CNV secondary to SFD after treatment with argon laser (13). PDT treatment in combination with intravitreal triamcinolone acetonide has also found to be ineffective (14), whereas intravitreal or systemic Bevacizumab was found to be effective (9, 15). Balaskas et al (10) described a case of a patient with CNV secondary to SFD, which was successfully treated with

intravitreal ranibizumab (10). However, there is a lack of evidence of Sorsby patients treated with anti-VEGF therapy.

In S1, both eyes were affected by SFD. CNV involved the right eye 4 years before the left eye developed the first signs of CNV. Active CNV was defined by the presence of subretinal fluid (SRF), intraretinal fluid (IRF), macular haemorrhage and increased hyperfluorescent leakage on fundus fluorescein angiography (FFA). In S1, the right eye showed CNV 1st and was treated with Avastin IVIs on a PRN basis until sub-foveal fibrosis meant further treatment was futile. The left eye showed CNV activity for the 1st time in January 2009. Bevacizumab IVIs on a PRN basis were given until April 2015 when T&E therapy was initiated. Of note, since switching to T&E treatment protocol, the patient has had no recurrence of CNV.

The younger sister developed CNV in 2012, i.e. 5 years later after her initial presentation at the Southampton Eye Unit. At that time, it was possible to have NHS anti-VEGF treatment without significant delay.

We would like to highlight two observations that might generate further arguments in favour of a PROACTIVE regime such as Treat and Extend. Firstly, OCT-A was able to detect the presence of active CNV in the absence of SRF in the corresponding OCT scan. Thus, adopting a PROACTIVE Treat and Extend regimen, even in the absence of fluid, would protect the eye from recurring disease contrary to what would happen with a PRN approach. Secondly, despite close observation a PRN approach did not stop visual loss due to progressive macular scarring. In contrast, our patients have been stable since switching to a treat and extend protocol.

Interestingly CNV activity in the younger sister (S2) started earlier compared to her elder sister (S1). One potential explanation is the fact that she was pregnant when she developed CNV. Hormonal changes occurring during pregnancy might have accelerated the development of CNV. This is consistent with observations in another case series (16).

The elder sister developed Bevacizumab-associated uveitis during her course of treatment with Bevacizumab injections. Bevacizumab-associated uveitis has been reported previously (17-19). Close monitoring is required to differentiate between a non-infectious uveitis and an endophthalmitis.

PRN or as needed treatment regimen is a historic treatment protocol that used to be and in some NHS trusts is still the applied regimen of choice not only for CNV secondary to hereditary macular conditions but also for CNV secondary to AMD. T&E, a pattern of treatment first suggested by Spaide and colleagues (20), is gaining popularity in the UK with supporting evidence of its effectiveness (21).

A PROACTIVE treatment regimen appears more effective than a PRN protocol at maintaining visual acuity gains and preventing deterioration of vision in age related macular degeneration (20, 21). We also observed this in S1, where stabilization of vision was observed on switching to a T&E protocol (Figures 1-3).

T&E therapy was initiated for S2, however unfortunately her disease recurred when treatment was extended beyond 4 weeks.

Both sisters were further investigated for systemic abnormalities. Bone densitometry scans and lung function tests were performed. Both sisters had normal bone density scans and lung function tests. These tests were performed as TIMP-3 knock-out mice develop osteoporosis (22) and also bronchial malformations (23). Lung problems have also been reported in human SFD patients (24).

Interestingly, OCT-A in the younger sister demonstrated CNV undetectable by other imaging modalities (Figure 5). This further emphasises that a more aggressive treatment protocol is likely to be more effective in treating CNV secondary to SF. This observation has been described in a case report of a patient with SFD by Mohla et al (25).

Our study is limited by its retrospective nature and small number of patients. However, its strength is the length of follow up in these molecularly confirmed SFD patients. To the best of our knowledge, this is the first report of an attempt of T&E care protocol in such patients and its comparison with PRN protocol. At the moment, these are the only patients with CNV secondary to SFD receiving treatment in our department with a long follow up period (12 years) that would permit a comparison between different treatment protocols of anti-VEGF delivery. We advocate that a PROACTIVE Treat and Extend protocol might be of great benefit not only for idiopathic CNV but also for CNV secondary to hereditary conditions as is the case with SFD. However, a larger number of patients is required in order to support our hypothesis further.

Acknowledgements/ Conflicts of Interest

We thank the patients for their participation in this work. The authors declare no conflicts of interests and no funding was obtained for this work.

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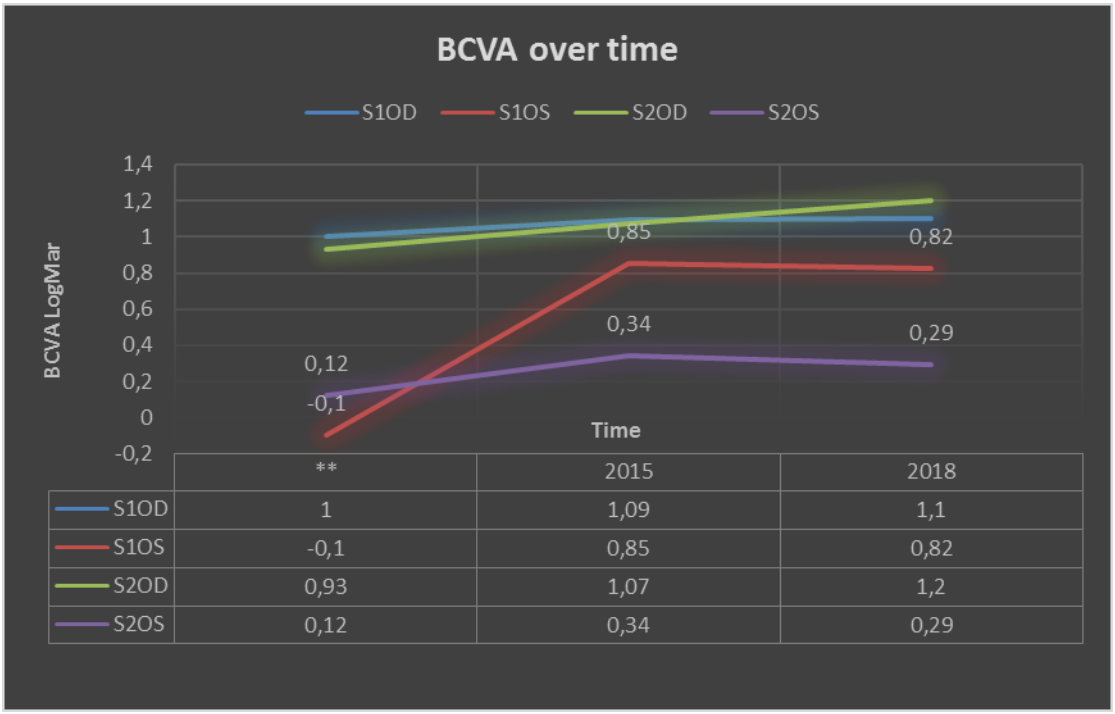
Patient	Mutation	Family History	Current Age (Years)	Age at onset (Years)	Initial Symptoms	Phenotype at last follow-up visit OD/OS
S1	TIMP-3 S204C Exon 5	Sister, Father, Paternal Aunt, Paternal Grandmother, Paternal Great Grand father	45	34	VA decline in right eye	OD: Disciform Scar OS: CNV with exudative maculopathy and fibrosis
S2	TIMP-3 S204C Exon 5	Sister, Father, Paternal Aunt, Paternal Grandmother, Paternal Great Grand father	42	36	Metamorphopsia	OD: CNV with fibrovascular PED OS: CNV with intraretinal fluid

Table 1 Summary of the genetic, phenotypic features and initial symptoms in both eyes for both sisters. S1: Sister 1. S2: Sister 2. VA: Visual Acuity. OD: Right Eye. OS: Left Eye.

Patient	VA at referral OD/OS (LogMar)	VA at first presentation OD/OS (LogMar)	Number of injections OD/OS	Number of PDT Sessions OD/OS	Treatment Modalities OD/OS	Mean VA ± SD (LogMar) OD/OS	Mean CRT ± SD (Microns) OD/OS
S1	0.2/-0.1	*1.0/-0.1	5/79	0/1	Right: PRN Left: PRN until April 2015 when switched to T&E	Right: 1.10 ± 0.17 Left: 0.82 ± 0.32	Right: 187.05 ± 30.74 Left: 223.81 ± 26.72
S2	-0.1/0.0	*0.93/ 0.12	24/42	None	Right: PRN Left: PRN until April 2015 when switched to T&E	Right: 1.20 ± 0.37 Left: 0.29 ± 0.21	Right: 242.67 ± 31.76 Left: 222.70 ± 31.41

Table 2 BCVA at different time points, number of IVIs, patterns of anti-VEGF treatment of both eyes of the two sisters. S1: Sister 1. S2: Sister 2. VA: Visual

Acuity. OD: Right Eye. OS: Left Eye. PRN: Pro Re Nata. T&E: Treat-and-Extend. SD: Standard Deviation. CRT: Central Retinal Thickness. *: Significant decline of VA between time of referral and time of 1st presentation at the Eye Unit in less than 2 weeks indicating the importance of rapid referral and initiation of anti-VEGF treatment in CNV secondary to SFD.



	**	2015	2018
S1OD	1	1,09	1,1
S1OS	-0,1	0,85	0,82
S2OD	0,93	1,07	1,2
S2OS	0,12	0,34	0,29

Figure 1 BCVA versus time. The importance of a PROACTIVE treatment regimen can be observed as from the point of switching to a treat and extend protocol at year 2015. The BCVA of the left eye in both patients stabilized with flattening of both curves (S1OS and S2OS) **: Presenting BCVA is different between eyes: S1OD-2006, S1OS-2009, S2OD-2012, S2OS-2013.

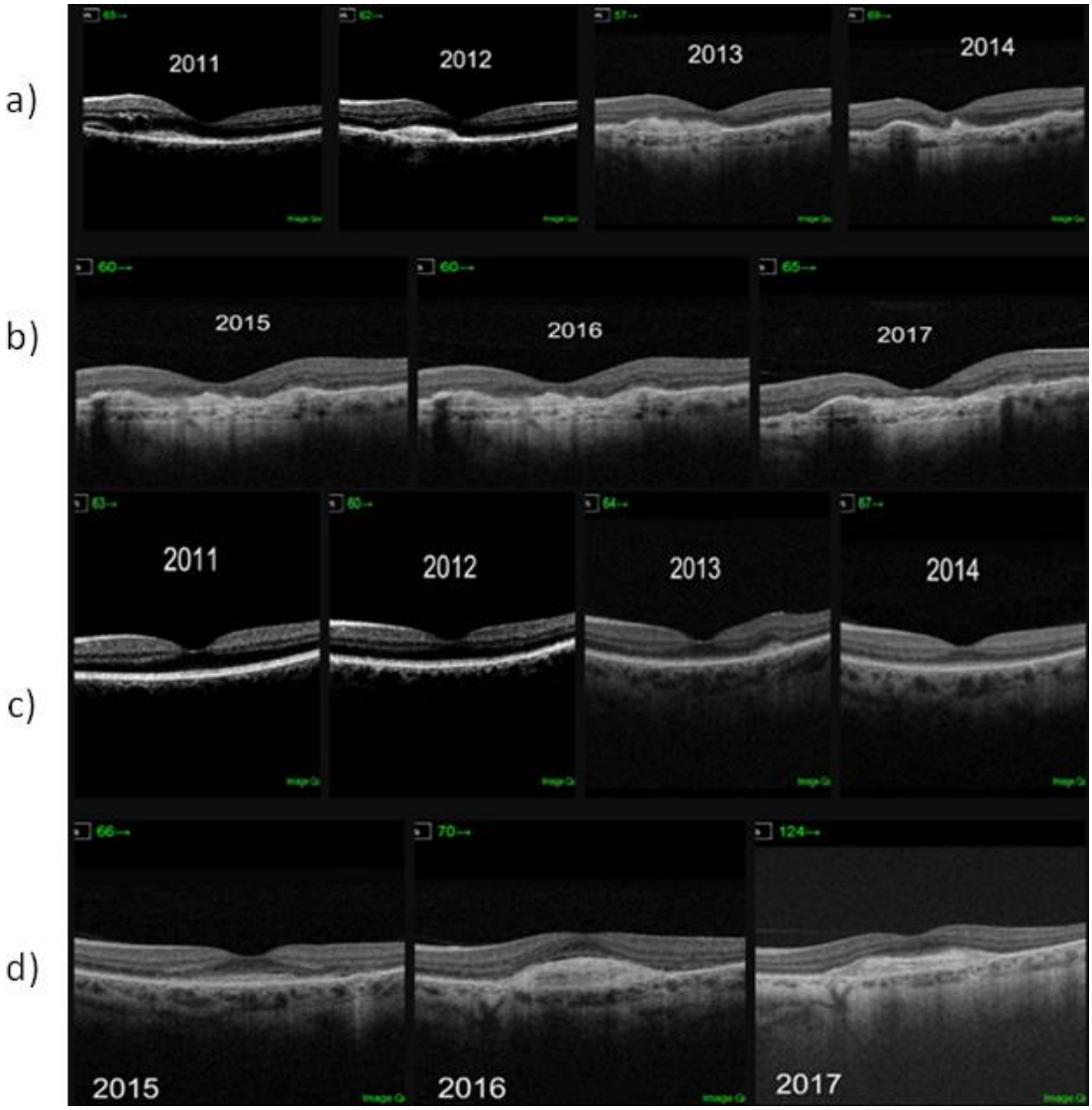


Figure 2.a,b) Top rows: Serial OCT Images of older sister's (S1) left macula. Note progression of fibrosis over the course of seven years after long-term course of Bevacizumab Intravitreal injections.

c,d) Bottom two rows: Serial OCT Images of younger's sister's (S2) left macula. Note the progression of subfoveal fibrosis over the course of 6 years after long-term course of Bevacizumab Intravitreal injections

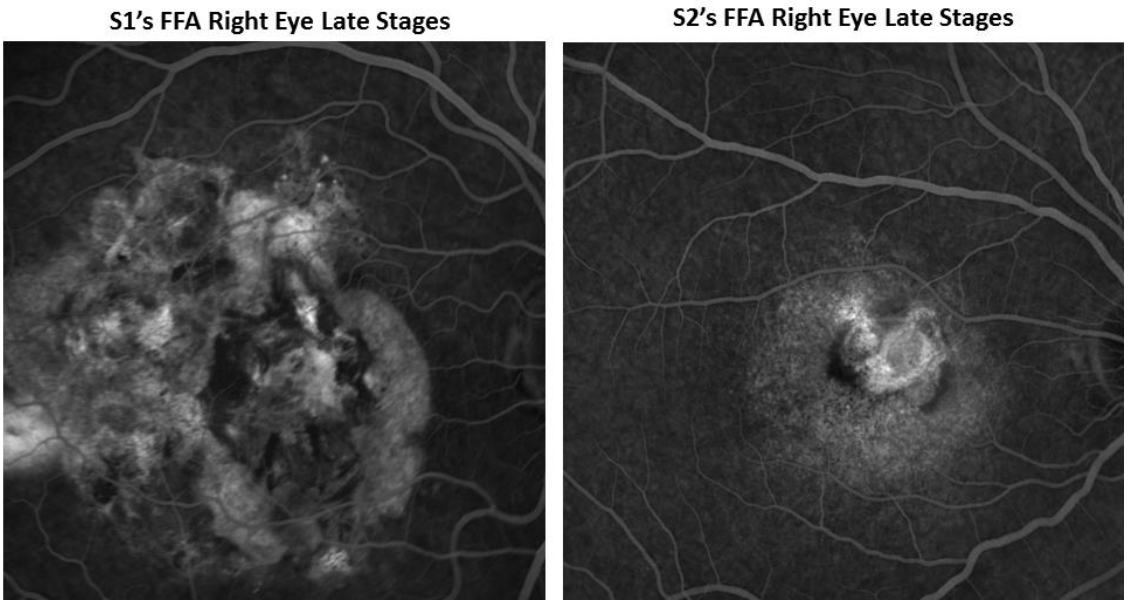


Figure 3. FFA from the right maculae of S1 and S2. Note the FFA revealed absence of leakage in early stages but late staining due to extensive fibrosis and scarring in the right eye of both S1 and S2.

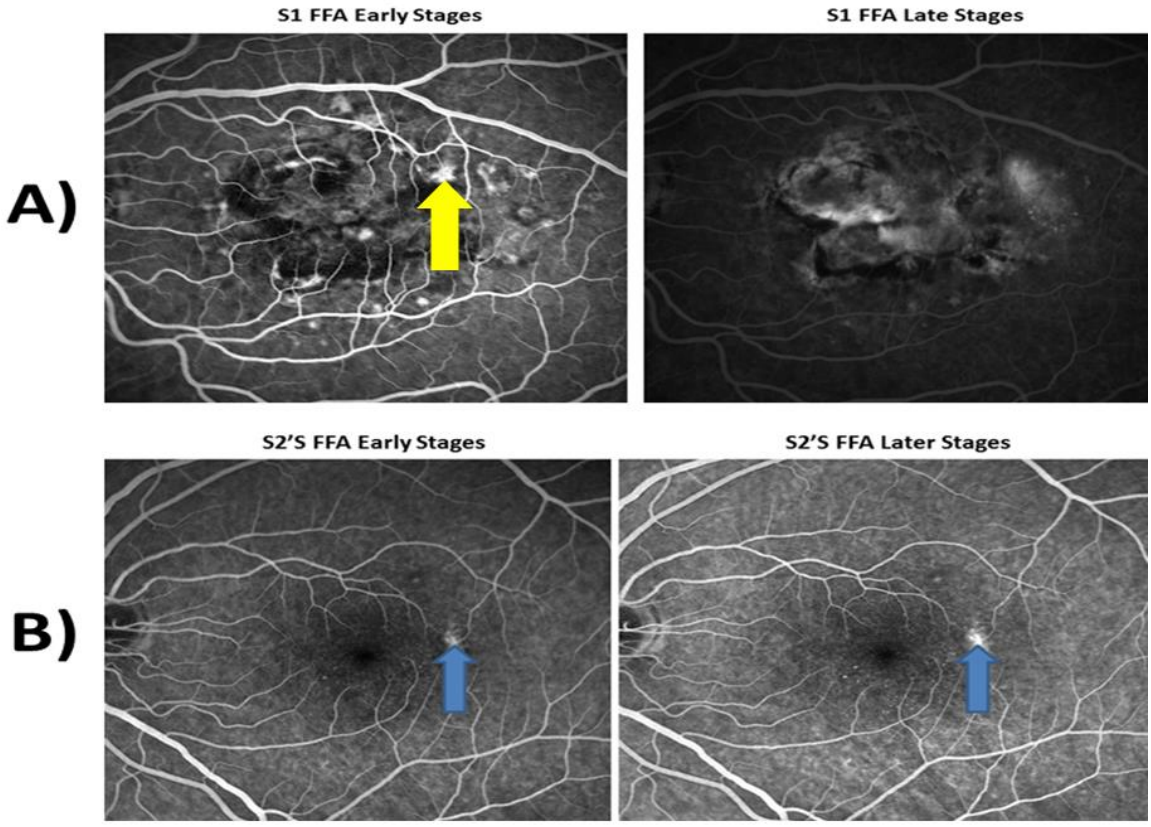


Figure 4. FFA from the left maculae of S1 and S2. A) Top Row: S1's FFA revealed a focal point of leakage in early stages (thick yellow arrow) and late hyperfluorescence due to late staining. There are also areas of hypofluorescence due to atrophy. B) Bottom Row: S2's FFA revealed a focal point of leakage growing in terms of intensity and size (thick blue arrows). This suggests an active CNV despite PRN treatment.

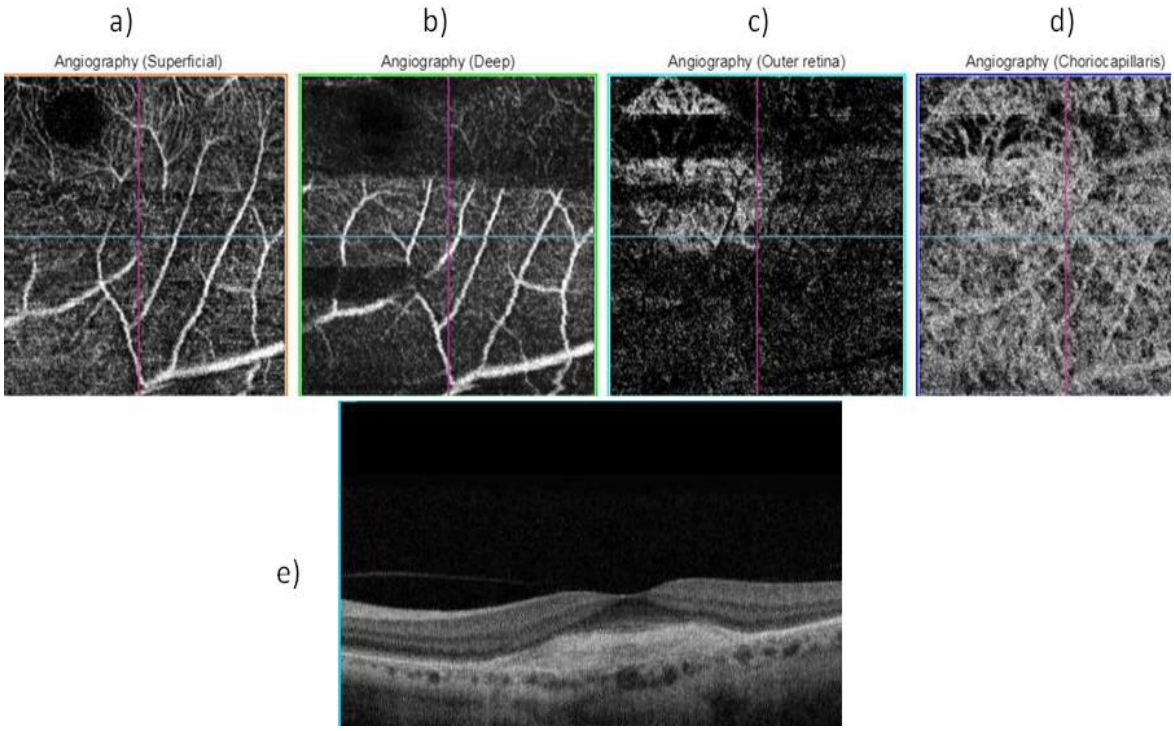


Figure 5. OCT-A from the younger sister's (S2) left macula. Top Row: a,b) Superficial and deeper vascular plexus of the inner retinal layers are both intact. Top Row: c,d) Evidence of an inactive CNV originating from the choriocapillaris and extending into the outer retinal layers, which was undetectable with other imaging modalities. Bottom row: e) The corresponding macula OCT scan of the left eye is also attached showing that there is no fluid on the OCT scan on the day of her last follow-up.

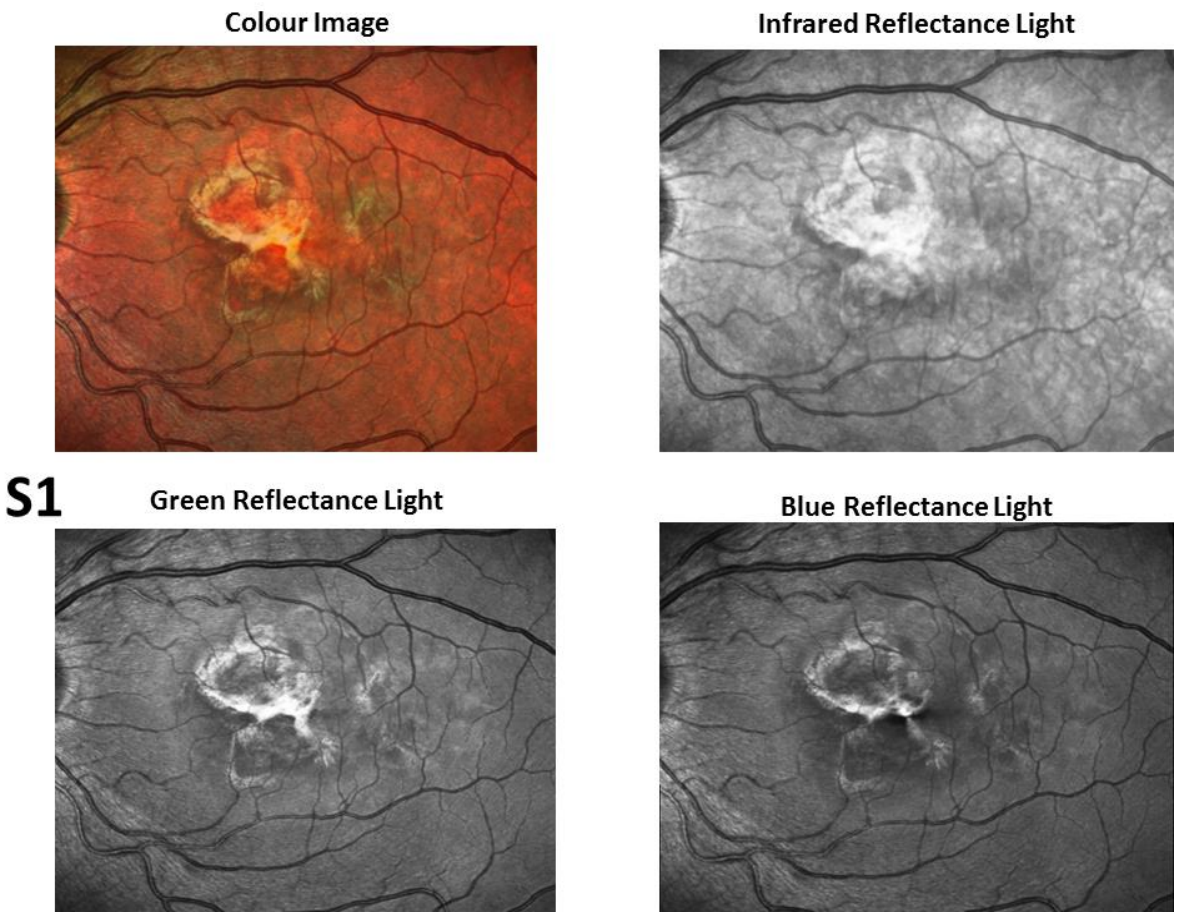
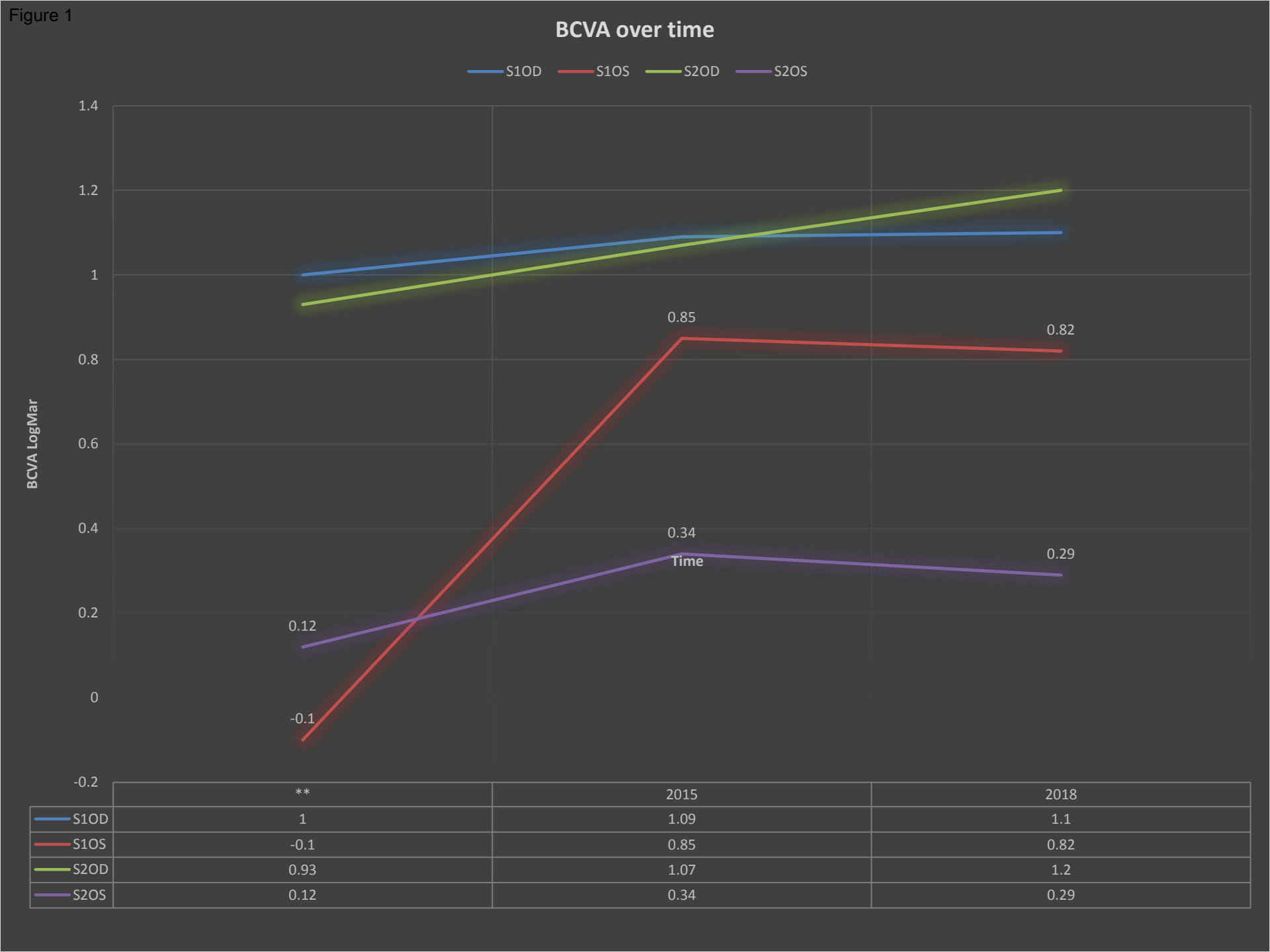


Figure 6. S1's multicolour imaging after treatment with IVI Bevacizumab.

Figure 1



	**	2015	2018
S1OD	1	1.09	1.1
S1OS	-0.1	0.85	0.82
S2OD	0.93	1.07	1.2
S2OS	0.12	0.34	0.29

Figure 2

Click here to access/download;Figure;Figure 2.tif

Figure 3

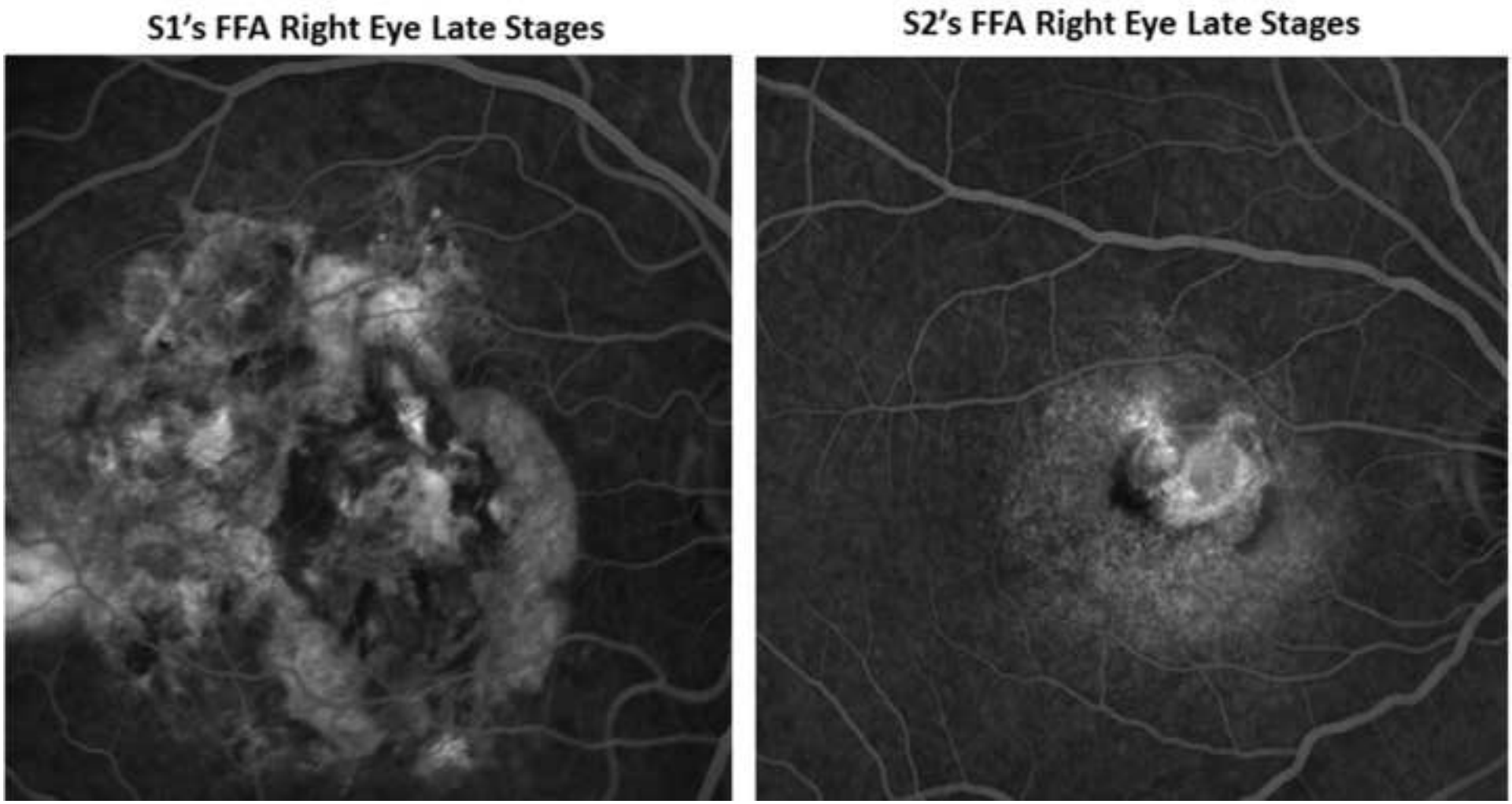


Figure 4

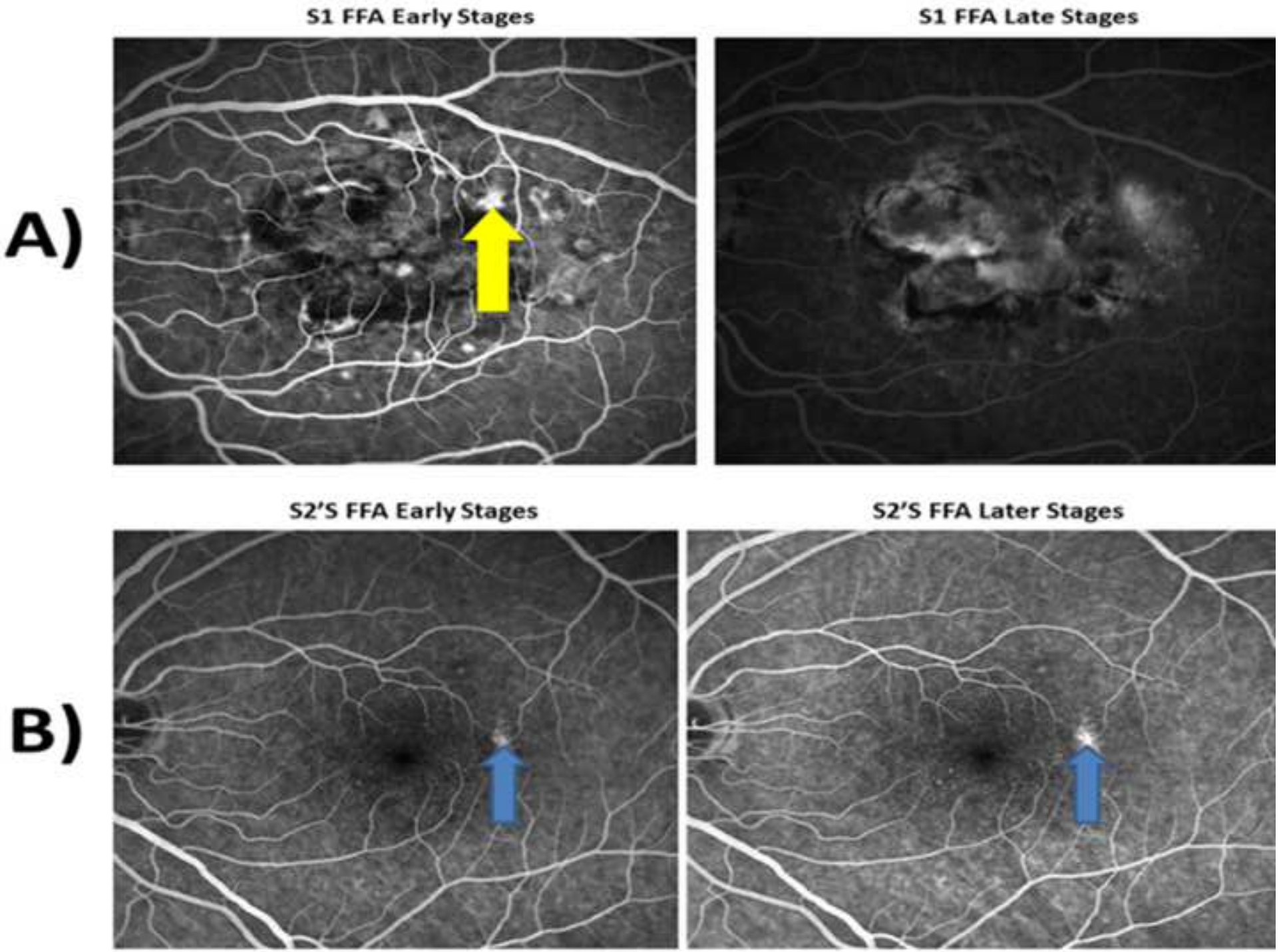


Figure 5

Click here to access/download;Figure;Figure 5.tif

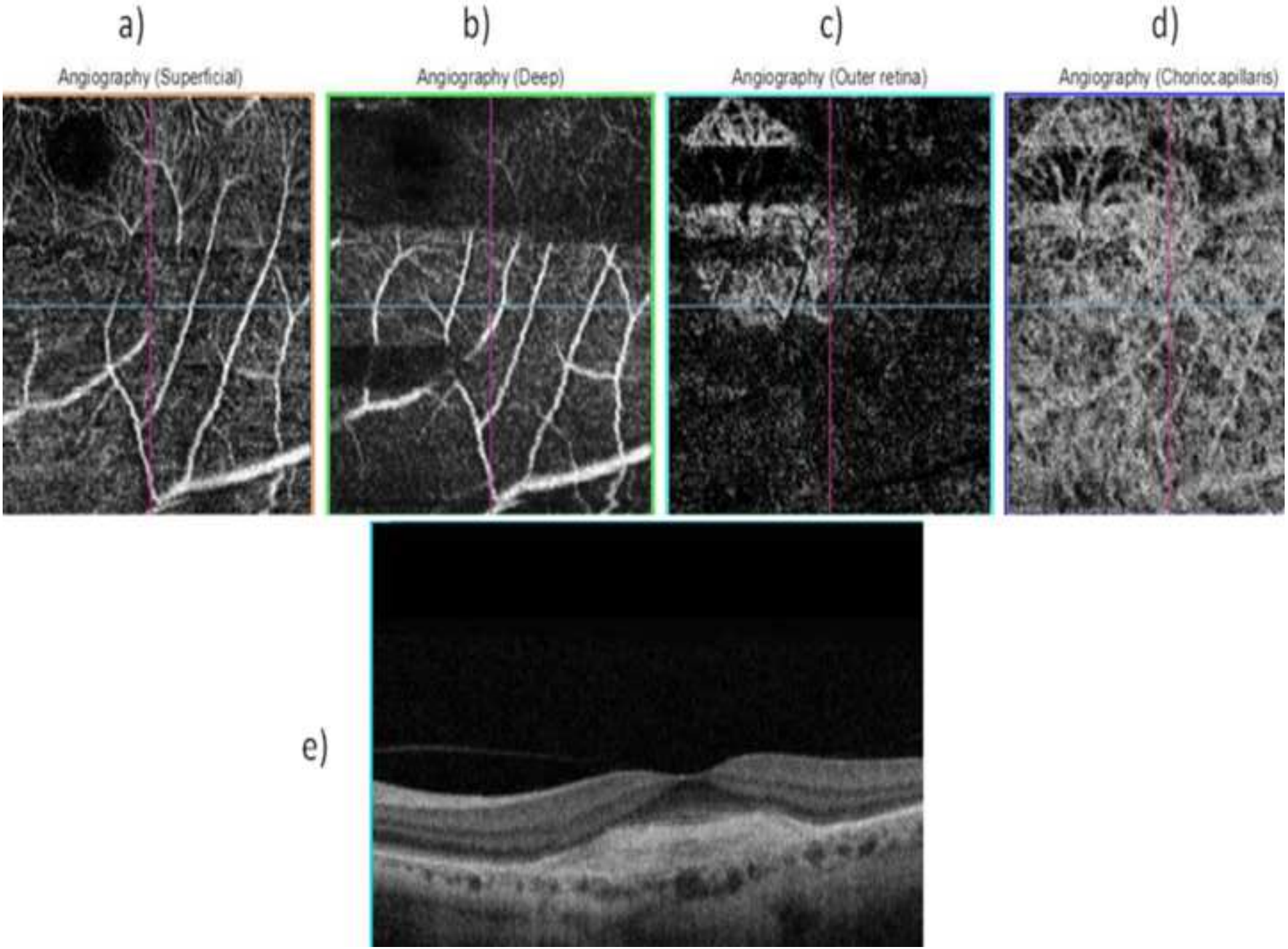


Figure 6