Fundus autofluorescence imaging: systematic review of test accuracy for the diagnosis and monitoring of retinal conditions

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Abstract

We conducted a systematic review of the accuracy of fundus autofluorescence (FAF) imaging for diagnosing and monitoring retinal conditions. Searches in November 2014 identified English language references. Sources included MEDLINE, EMBASE, the Cochrane Library, Web of Science, and MEDION databases; reference lists of retrieved studies; and internet pages of relevant organisations, meetings, and trial registries. For inclusion, studies had to report FAF imaging accuracy quantitatively. Studies were critically appraised using QUADAS risk of bias criteria. Two reviewers conducted all review steps. From 2240 unique references identified, eight primary research studies met the inclusion criteria. These investigated diagnostic accuracy of FAF imaging for choroidal neovascularisation (one study), reticular pseudodrusen (three studies), cystoid macular oedema (two studies), and diabetic macular oedema (two studies). Diagnostic sensitivity of FAF imaging ranged from 32 to 100% and specificity from 34 to 100%. However, owing to methodological limitations, including high and/or unclear risks of bias, none of these studies provides conclusive evidence of the diagnostic accuracy of FAF imaging. Study heterogeneity precluded meta-analysis. In most studies, the patient spectrum was not reflective of those who would present in clinical practice and no studies adequately reported whether FAF images were interpreted consistently. No studies of monitoring accuracy were identified. An update in October 2016, based on MEDLINE and internet searches, identified four new studies but did not alter our conclusions. Robust quantitative evidence on the accuracy of FAF imaging and how FAF

images are interpreted is lacking. We provide recommendations to address this.

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Introduction

Fundus autofluorescence (FAF) is increasingly being used in ophthalmology for detecting or monitoring retinal pathology, since autofluorescent molecules, referred to as fluorophores, can provide potentially useful diagnostic and prognostic information.¹ Extensive and focal accumulations of lipofuscin and other fluorophores usually indicate retinal pathology^{1,2} and result in patterns of increased (hyper) autofluorescence relative to those seen in a normal eye. In conditions involving atrophy of the retina, (eg, geographic atrophy) or where morphological changes, fluid accumulation, fibrosis, pigment changes, or haemorrhages obscure autofluorescence, decreased (hypo) autofluorescence may also signal retinal pathology.^{3,4} As a result, characteristic spatial distributions of hyper-autofluorescence and hypo-autofluorescence can be associated with a wide range of retinal conditions.¹

The intensity of FAF is relatively weak (about two orders of magnitude lower than the background emission of a fluorescein angiogram at peak dye transit),³ meaning that specialist imaging techniques are required to enable FAF to be detected and mapped. Three main techniques have been used for FAF imaging, which are: fundus camera, fundus spectrophotometry, and confocal scanning laser

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ophthalmoscopy (cSLO).^{1,3} Compared to the other methods, cSLO is superior at minimising the detection of artefacts such as autofluorescence arising from structures anterior to the retina (eg, the cornea or lens).⁴ As such, cSLO is considered the most appropriate method currently available for measuring FAF. In cSLO, a focused low-power laser beam is swept across the fundus in a raster pattern to provide the excitatory light source for fluorophores.⁵ Excitation wavelengths include 488 nm (blue light) obtained with a solid-state laser and, developed more recently, 514 nm (green light) with an argon-ion laser.⁴ For near-infrared FAF, the excitation wavelength is 790 nm. Unlike fundus spectrophotometry, cSLO can cover relatively large retinal areas (eg, 55° in Heidelberg retina angiograph (HRA)-based systems). A FAF image obtained from cSLO typically shows the spatial distribution of FAF intensity as pixels in greyscale values with dark pixels indicating low FAF intensity and bright pixels indicating high FAF intensity.³ The mean image of several FAF images is usually obtained to reduce background noise and enhance image contrast. To block reflected light but permit autofluorescence to pass, cSLO have barrier filters of 500, 525 and 800 nm for excitation wavelengths of 488, 514 and 790 nm, respectively. Image contrast and brightness settings also differ among cSLO devices and these differences must be taken into account when comparing the results of FAF imaging obtained from different cSLO devices.³

Numerous studies suggest that FAF imaging could assist the detection and/or monitoring of various retinal conditions,^{1,3} for example, geographic atrophy,^{4,6–12} choroidal neovascularisation development in age-related macular degeneration (AMD),^{13–17} retinal pigment epithelium alterations,^{14,18–22} retinitis pigmentosa,^{7,23–30} Best's disease,^{2,31–34} Stargardt disease,^{2,34–37} cystoid macular oedema,^{38–43} diabetic macular oedema,^{44–49} and central serous chorioretinopathy,^{14,50–58} among others. Assessment of FAF has also been specified as an outcome in clinical research studies of therapies for retinal conditions, for example, with anti-vascular endothelial growth factor drugs,^{18,59–61} other drugs,^{9,62,63} photodynamic therapy,⁶⁴ and vitrectomy.⁶⁵ However, despite this extensive interest in FAF imaging, these studies have not hitherto been critically assessed and it is unclear how much robust quantitative evidence exists to support the accuracy of FAF imaging using cSLO, that is, its sensitivity and specificity, for the diagnosis and/or monitoring of retinal conditions.

To determine the accuracy of FAF imaging using cSLO for the diagnosis and/or monitoring of retinal conditions, we conducted a systematic review of studies in a wide range of retinal conditions. Full details of the review are reported by Frampton *et al.*⁶⁶ Here, we summarise the key methods and findings of the systematic review, and a

review update conducted in October 2016, and we consider the implications of the current evidence for the application of FAF imaging to detect and/or monitor retinal conditions in clinical practice.

Materials and methods

The methods for this systematic review were specified *a priori* in a protocol⁶⁷ that was subjected to independent peer review.

Identification of evidence

A comprehensive search strategy was developed and refined by an experienced information specialist to identify studies on the diagnosis and/or monitoring of retinal conditions based on the following pre-specified inclusion and exclusion criteria:

- Population: of any age with any retinal condition, excluding those resulting from malignancy, or those secondary to major ocular conditions (eg, glaucoma) or trauma.
- Index test: FAF imaging performed using cSLO.
- Reference standard: fundus imaging performed using any clinically relevant method (eg, fundus photography, fundus fluorescein angiography, indocyanine green angiography, optical coherence tomography, or any combination of relevant tests).
- Primary outcomes: sensitivity and/or specificity for the diagnosis or monitoring of retinal conditions.
- Secondary outcomes (applicable only if primary outcomes were also reported): inter- and intra-observer agreement, adverse events, test acceptability to patients and clinicians, and test interpretability.
- Study designs: any study design, provided that the index test(s) and reference standard were compared in the same patient group and the sample size was ≥ 10 eyes per group.

Searches were conducted in the following electronic bibliographic databases: MEDLINE; PreMedline In-Process and Other Non-Indexed Citations; EMBASE; the Cochrane Library; Web of Science, including the Science Citation Index-Expanded (SCI-E) and the Conference Proceedings Citation Index-Science (CPCI-S); Database of Abstracts of Reviews of Effectiveness (DARE); Health Technology Assessment Database (HTA); NHS Economic Evaluation Database (NHSEED); and the MEDION database of diagnostic accuracy studies. In addition, searches were made in internet pages of the American Academy of Ophthalmology (AAO); Association for Research in Vision and Ophthalmology

(ARVO); Cochrane Eyes and Vision Group (CEVG); European Association for Vision and Eye Research (EVER); and the Royal College of Ophthalmologists. Trial registries searched were the Cochrane Central Register of Controlled Trials, UK Clinical Research Network Portfolio Database; World Health Organization International Clinical Trials Registry Platform (WHO ICTRP); Current Controlled Trials; ClinicalTrials.gov; and NIHR Clinical Research Network Portfolio. In addition, bibliographies of retrieved papers and relevant systematic reviews were searched. All searches were conducted for the period from 1990 (~10 years prior to the likely publication of the earliest relevant evidence) to November 2014 and searches were limited to the English language.

Search update

The current paper was submitted for publication 20 months after our original literature search. To check whether our conclusions remain applicable, we conducted an updated search in October 2016. Limited team availability precluded us re-running the full searches described above. Instead, we re-ran our original MEDLINE search,⁶⁶ and supplemented this with a broad search in Google Scholar using the free text term 'fundus autofluorescence imaging'. The first 200 potentially relevant references identified in Google Scholar, as judged by one reviewer against the inclusion criteria, and all references identified in the MEDLINE search were imported into an Endnote library. As there were differences in the methods of the original and update searches, we present the results obtained from the two searches separately below.

Selection of evidence

Titles and abstracts identified in the original searches were screened independently by two reviewers against the inclusion criteria to identify potentially relevant bibliographic records. Full-text articles were retrieved for those records judged to be relevant or unclear at the title and abstract screening step. Full-text articles were screened by one reviewer and checked by a second reviewer. At both steps of the selection process, a standard study selection worksheet listing the inclusion criteria was employed,⁶⁶ and any disagreements were resolved by discussion among the two reviewers or, if necessary, by involving a third reviewer. If a study was reported in more than one article at the full-text assessment step, all the articles relating to the study were grouped together for the eligibility assessment.

The study selection process for the search update was identical to that of the original search except that the title and abstract screening step was conducted by one

reviewer. A randomly selected subset of 20% of the titles and abstracts identified in the search update was checked by a second reviewer, with 100% reviewer agreement (Supplementary Appendix A).

Data extraction, critical appraisal, and data synthesis

The data extraction, critical appraisal, and data synthesis steps of the original systematic review were conducted by one reviewer and checked by a second reviewer. Any disagreements were resolved through discussion between the reviewers. For the review update, these steps were conducted by one reviewer. The update search results are illustrative and as such are reported separately from those of the original review below.

Data were extracted from the included studies using a pre-designed and pilot-tested data extraction form⁶⁶ to minimise errors. If test accuracy outcomes were not fully reported, missing values were calculated by the reviewers where possible.

Methodological rigour of the included studies was assessed using the Cochrane Collaboration adaptation⁶⁸ of the QUADAS tool,⁶⁹ which asks 11 questions about the characteristics of the primary studies. These questions aim to identify potential threats to the validity of the study findings and (in our interpretation) reflect 10 different types of bias that may occur in studies of test accuracy. We have interpreted the QUADAS questions to determine whether for each of the included studies there is a low, high, or unclear risk of each type of bias (Supplementary Table 1 provides an explanation of the QUADAS questions and the types of bias being assessed).

The method of data synthesis allowed for meta-analysis of test accuracy outcomes if studies were sufficiently similar, as specified in the review protocol.⁶⁷

Results

As there were some differences in the methods of our original systematic review and the review update, results of the review update are presented separately below.

Identification and characteristics of studies

Searches conducted in November 2014 yielded 2240 unique bibliographic records, of which 2034 (91%) were excluded upon inspection of the title and abstract (Figure 1). Of 206 bibliographic records obtained for further scrutiny, 198 (96%) were excluded, primarily because 163 (85%) did not report (or provide sufficient data to enable us to calculate) sensitivity and/or specificity of FAF imaging. Eight research publications reporting eight unique studies met the eligibility criteria and were included in the systematic review.^{39,41,48,70–74}

Figure 1 Flow chart for the identification of studies.

Key characteristics of the included studies are summarised in Supplementary Table 2 and are given in detail in the full study report.⁶⁶ Although FAF imaging may be used to detect autofluorescence in a wide range of retinal conditions, the eight included studies cover only choroidal neovascularisation in AMD,⁷⁰ reticular pseudodrusen in AMD,^{71–73} cystoid macular oedema,^{39,41} and diabetic macular oedema.^{48,74} Six of these studies were European, one was conducted in the United States,⁷² and one in Japan.⁷³ The number of participants ranged from 34 to 138 and the number of eyes from 34 to 263. None of the studies involved people younger than 17 years of age, and the mean age ranged from 49 to 76 years, although not all studies gave this information. The studies differed in whether they admitted participants with any ocular comorbidity besides the retinal condition of primary interest. Despite the extensive research interest noted above in the use of FAF imaging for monitoring the course of retinal conditions and their response to therapy, none of the included studies investigated FAF imaging for monitoring disease progression or response to therapy. The study designs were either prospective or retrospective cohorts, and in all cases the unit of analysis was the eye. However, the studies differed according to whether they included one eye per patient, both eyes, or a mixture of single eyes and both eyes (Supplementary Table 3). Furthermore, the studies on reticular pseudodrusen in AMD^{71–73} differed according to the stage of AMD, and whether it affected one or both eyes; the studies on cystoid macular oedema^{39,41} differed in the

primary conditions from which the cystoid macular oedema developed; and the studies on diabetic macular oedema^{48,74} differed as to whether they included ocular comorbidities. Because of this extensive heterogeneity among the studies, we considered it inappropriate to conduct a meta-analysis.

The studies (where reported) used variants of the HRA cSLO for obtaining FAF images (ie, the index test), primarily employing an excitation wavelength of 488 nm (blue), with two studies additionally including a 514 nm (green) wavelength⁴⁸ and a 790 nm (near-infrared) wavelength⁷³ (Table 1). The comparator tests (ie, reference standards) included various types of colour fundus photography, fluorescein angiography, scanning laser ophthalmoscopy, and optical coherence tomography, although two studies^{71,73} employed comparisons in which a positive result was required on one of five⁷¹ or two of seven⁷³ imaging tests. The diagnostic criteria on FAF imaging for each of the retinal conditions were qualitative (ie, descriptive) in all studies, and only one of the studies reported an objective (quantitative) approach for determining how abnormal (hypo or hyper) autofluorescence was defined.⁷² The three studies on reticular pseudodrusen all specified that the diagnostic criterion was a reticular pattern of hypo-autofluorescence against a background of mildly increased autofluorescence, although there were differences in how this was described in each study.^{71–73} The two studies on cystoid macular oedema^{39,41} both appeared to diagnose the condition as round or oval patterns of autofluorescence at the fovea, although this was not stated explicitly in one study.³⁹ The two studies on diabetic macular oedema^{48,74} only mentioned the diagnostic criteria briefly and differed in how these were described.

Risks of bias

Assessment of the risk of bias (Table 2) identified two main problems with the studies (the decision criteria are shown in Supplementary Table 1). First, seven studies were deemed to be at high risk and one at unclear risk of spectrum bias, since the population with retinal disease was not representative of those who would receive a FAF imaging test in clinical practice. Two studies on reticular pseudodrusen in AMD were also deemed to be at high risk of biases related to the use of an inappropriate reference standard (differential verification bias and incorporation bias).^{71,73} The second problem is that, for many domains of bias that were assessed, the risk of bias was unclear due to a lack of adequate reported methodological information (unclear risk of bias means that the possibility of high risk of bias could not be ruled out). Notably, all the studies were deemed to be at unclear

Table 1 Diagnostic accuracy of FAF imaging

<i>Retinal condition</i>	<i>Study</i>	<i>Index text</i>	<i>Reference standard</i>	<i>Sensitivity% (95% CI)</i>	<i>Specificity% (95% CI)</i>
Choroidal neovascularisation in AMD	Cachulo <i>et al</i> ⁷⁰	FAF 488 nm	Fluorescein angiography	88 ^a (64–98) ^a	34 ^a (19–52) ^a
Reticular pseudodrusen in AMD	Smith <i>et al</i> ⁷²	FAF ^b	Colour fundus photography	93 (78–99)	98 (95–99)
Reticular pseudodrusen in AMD	Hogg <i>et al</i> ⁷¹	FAF ^b	Spectralis OCT	88 (72–97)	84 (72–92)
Reticular pseudodrusen in AMD	Hogg <i>et al</i> ⁷¹	FAF ^b	Colour fundus photography	100 (78–100)	67 (55–77)
Reticular pseudodrusen in AMD	Hogg <i>et al</i> ⁷¹	FAF ^b	Positive on ≥ 1 of 5 imaging modalities (CFP, RFP, infrared photography, FAF, and OCT)	95 (84–99)	100 (93–100)
Reticular pseudodrusen in AMD	Ueda-Arakawa <i>et al</i> ⁷³	FAF 488 nm	Positive on ≥ 2 of 7 imaging modalities (CFP, IRR, FAF, NIR-FAF, CBR, IGA, and spectral domain OCT)	86 (71–95)	95 (91–98)
Reticular pseudodrusen in AMD	Ueda-Arakawa <i>et al</i> ⁷³	FAF 790 nm	Positive on ≥ 2 of 7 imaging modalities (CFP, IRR, FAF, NIR-FAF, CBR, IGA, and spectral domain OCT)	32 (16–52)	95 (90–98)
Cystoid macular oedema	Dinc <i>et al</i> ³⁹	FAF 488 nm	Fluorescein angiography	98 (92–100)	0 (0–81)
Cystoid macular oedema	McBain <i>et al</i> ⁴¹	FAF 488 nm	Fluorescein angiography	81 (58–94)	69 (39–91)
Diabetic macular oedema	Vujosevic <i>et al</i> ⁷⁴	FAF 488 nm	Retromode scanning laser ophthalmoscopy	92 (88–96)	85 (72–93)
Diabetic macular oedema	Waldstein <i>et al</i> ⁴⁸	FAF 488 nm	Spectral domain OCT	81 (69–89)	90 (79–96)
Diabetic macular oedema	Waldstein <i>et al</i> ⁴⁸	FAF 514 nm	Spectral domain OCT	55 (43–67)	95 (86–99)

Abbreviations: AMD, age-related macular degeneration; CBR, confocal blue reflectance; CFP, colour fundus photography; CI, confidence interval; FAF, fundus autofluorescence; IGA, indocyanine green angiography; IRR, infrared reflectance; NIR-FAF, near-infrared fundus autofluorescence (790 nm); OCT, optical coherence tomography; RFP, red fundus photography. ^aCalculated by reviewer from data reported in the study publication. ^bExcitation wavelength not reported.

Table 2 Risks of bias identified in the included studies

QUADAS question	Bias type	CNV in AMD	Reticular pseudodrusen in AMD		Cystoid macular oedema		Diabetic macular oedema		
		Cachulo et al ⁷⁰	Smith et al ⁷²	Hogg et al ⁷¹	Ueda-Arakawa et al ⁷³	Dinc et al ³⁹	McBain et al ⁴¹	Vujosevic et al ⁷⁴	Waldstein et al ⁴⁸
1	Spectrum bias	High	High	High	High	High	High	Unclear	High
2	Verification bias	Low	Low	Unclear	Unclear	Low	Low	Unclear	Low
3	Disease progression bias	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
4,5	Differential verification bias	Low	Low	High	High	Low	Low	Low	Low
6	Incorporation bias	Low	Low	High	High	Low	Low	Low	Low
7	Diagnostic review bias	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
8	Test review bias	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Unclear
9	Clinical review bias	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
10	Image classification bias	Unclear ^a	Unclear ^b	Unclear ^b	Low ^c	Unclear ^b	Low ^c	Unclear ^b	Unclear ^b
11	Attrition bias	Unclear ^d	Low ^e	Unclear ^f	Low ^g	Low ^e	Unclear ^d	Low ^e	Low ^e

^aNumber of poor quality images reported but unclear whether included in analysis. ^bNumber of uninterpretable (eg, poor quality) images not fully reported. ^cNumber of poor quality images reported and excluded from analysis. ^dInconsistency in numbers of withdrawals reported/analysed. ^eNo withdrawals during study. ^fWithdrawals not mentioned. ^gWithdrawals occurred but unlikely to influence results.

Table 3 Aspects of study methodology not covered in QUADAS criteria

Methodology aspect	CNV in AMD		Reticular pseudodrusen in AMD		Cystoid macular oedema		Diabetic macular oedema	
	<i>Cachulo et al</i> ⁷⁰	<i>Smith et al</i> ⁷²	<i>Hogg et al</i> ⁷¹	<i>Ueda-Arakawa et al</i> ⁷³	<i>Dinc et al</i> ³⁹	<i>McBain et al</i> ⁴¹	<i>Vijosevic et al</i> ⁷⁴	<i>Waldstein et al</i> ⁴⁸
Prospective study design	Yes	No	Yes	No	Yes	No	Unclear	No
Consecutive patient recruitment	Not reported	Not reported	Not reported	Yes	Not reported	Yes	Yes	Yes
Correlations between eyes accounted for	Not applicable	No	Not applicable	No	Not applicable	Not applicable	No	No
Inter-observer agreement reported	No	No	Yes, but only for one of three study centres	Yes	No	No	No	Yes
Intra-observer agreement reported	No	No	No	No	No	No	No	No
Test acceptability to patients or clinicians reported	No	No	No	No	No	No	No	No

risk of clinical review bias since none of them provided unequivocal statements about which clinical information, if any, was considered when interpreting the FAF images. In clinical practice, it is unlikely that FAF images would be interpreted in isolation of other clinical information, so it is possible that these studies may have systematically overestimated or underestimated the sensitivity and specificity of FAF imaging relative to how it would be applied in clinical practice.

Other aspects of study methodology

Several aspects of the study methodology that are not covered by QUADAS criteria could have implications for interpretation of the study findings (Table 3). Ideally, to minimise the risk of selection bias, the cohort studies should have been conducted prospectively and should have recruited participants consecutively.⁷⁵ However, as shown in Table 3, this is not clear in the publications of five of the eight studies and adds further uncertainty about the reliability of these studies' findings.

Four of the eight reviewed studies analysed both of the participants' eyes, and in such cases it would be appropriate to consider intra-subject correlations in the analysis.⁷⁶ However, these studies did not mention the correlation or make any adjustment for it. Although it is not clear that this would lead to bias, intra-individual correlation could lead to underestimation of the standard error, and hence the confidence intervals, for sensitivity and specificity estimates.

Despite the subjective nature of the diagnostic criteria, only three of the eight studies investigated inter-grader agreement in the classification of retinal images. Limited information was provided about the expertise of the image graders, who were described as two independent ophthalmologists,⁷³ two retinal specialists trained in image grading,⁷⁴ and two independent graders.⁴⁸ None of the studies reported intra-grader agreement (ie, a person's consistency of image interpretation), or whether the FAF imaging technique was considered acceptable by the participants or clinicians.

Accuracy of FAF imaging

As shown in Table 1, diagnostic sensitivity of FAF imaging compared to various reference standards ranged from 32 to 100% and specificity from 34 to 100%. The sensitivity was lowest when FAF emission wavelengths longer than 488 nm were used (32% for 790 nm⁷³ and 55% for 514 nm⁴⁸), although two studies with relatively good sensitivity (88–100%) did not specify the emission wavelengths they employed.^{71,72} Specificity was lowest (34%) for the study on choroidal neovascularisation in AMD⁷⁰ and one study on cystoid macular oedema (0%).³⁹ In the latter study, specificity was zero since no true-

negative results were identified when FAF imaging was compared against fluorescein angiography.³⁹ However, it is questionable whether any of these test accuracy findings are clinically meaningful, given their extensive methodological limitations, risks of bias, and the interpretational uncertainties mentioned above.

Results of the review update

Supplementary Appendix A provides results of the updated search in October 2016, including a PRISMA flow chart showing the study selection process; a list of studies excluded at full-text screening; the QUADAS risk of bias assessment; and an overview of the characteristics and results of the included studies. After removing duplicates, the updated search in October 2016 yielded 380 new references. Eighteen references were retrieved for screening on full text,^{77–94} of which four met the criteria for inclusion in the systematic review.^{79,80,82,91}

The four new references reported four unique studies. These investigated FAF imaging accuracy for detecting: reticular pseudodrusen in AMD (two studies^{80,91}); hydroxychloroquine-induced retinopathy (one study⁷⁹); and lacquer cracks in highly myopic eyes (one study⁸²). In two of these studies, the FAF imaging excitation wavelength was 488 nm;^{79,80} in one study, both 488 and 787 nm (near-infrared) wavelengths were employed; and one study did not report the excitation wavelength.⁹¹ The reference standards were: spectral domain optical coherence tomography in addition to one or more other imaging methods to detect reticular pseudodrusen;^{80,91} multifocal electroretinography to detect hydroxychloroquine-induced retinopathy;⁷⁹ and indocyanine green angiography to detect retinal lacquer cracks.⁸² In all studies, the unit of analysis was the eye and sample sizes ranged from 56 eyes⁸² to 600 eyes.⁹¹

Critical appraisal of the four new studies indicated that they exhibited similar methodological limitations to the studies included in our original review: all four studies were deemed to be at high risk of spectrum bias because they excluded patients with comorbidities and therefore would not be reflective of the case mix presenting in clinical practice; and all four studies were deemed to be at unclear risk of clinical review bias because it was not clear whether the clinical information available to FAF image readers was the same as that which would be available in clinical practice. The studies were judged to be at low or unclear risks of the other types of bias assessed by the QUADAS instrument.

Sensitivity of FAF imaging across these retinal conditions was: reticular pseudodrusen: 73%⁸⁰–89%;⁹¹ hydroxychloroquine-induced retinopathy: 80%;⁷⁹ and lacquer cracks: 12.5% with 488 nm FAF and 93% with 787 nm FAF.⁸² Specificity was 97–100% for reticular

pseudodrusen^{80,91} and 88% for hydroxychlorine retinopathy,⁷⁹ but not reported for retinal lacquer cracks.⁸² None of the four studies reported sufficient data for true-positive, true-negative, false-positive, or false-negative test results, or predictive values, to be calculated.

Discussion

Our systematic review highlights some key issues concerning the use of FAF imaging for detecting or monitoring retinal conditions in clinical practice. The academic literature shows that FAF imaging is used extensively for the detection and/or monitoring of a wide range of retinal conditions in research studies, but our critical examination of the evidence identified that only a minority of these studies have formally assessed the accuracy of the method, for very few retinal conditions. Moreover, despite the relatively high estimates of sensitivity and/or specificity reported in some studies, the methodological shortcomings of the studies, together with inadequate reporting of study details, leave us uncertain as to whether the results of this limited set of primary studies are reliable. It is also unclear whether the results of any of these research studies would be truly reflective of how FAF imaging tests would be interpreted if applied in clinical practice.

FAF imaging could have potential for diagnosing inherited retinal dystrophies such as retinitis pigmentosa, cone-rod dystrophies, and Stargardt disease, or for monitoring the progression of Best's disease,³² but our original systematic review and the update conducted in October 2016 did not identify any quantitative comparisons of test accuracy in these conditions.

In the present systematic review, we have followed the paradigm of test accuracy assessment,^{68,95,96} in which we have quantified the accuracy of FAF imaging (the index test) when compared against a comparator (the reference standard). The paradigm of test accuracy assessment requires that the reference standard should, preferably, be the 'gold standard', that is, identifying with optimal accuracy (ideally 100%) which of the patients tested have a specified retinal condition (true positives) and which do not have the condition (true negatives). However, since FAF imaging based on cSLO utilises confocal optics and filters to reduce optical artefacts and provide a high-resolution image, it is plausible that FAF imaging would be more accurate than existing imaging methods (eg, colour fundus photography) at detecting certain types of retinal pathology.⁹⁷ FAF imaging also detects different retinal processes compared to other imaging methods; for example, while fluorescein angiography detects leakage, FAF imaging detects metabolic activity.⁹⁷ For these reasons, there may not always be an obvious gold standard test against which to compare FAF imaging. Publications that have discussed the clinical relevance of

FAF imaging^{4,97,98} suggest that the benefits of FAF imaging have largely been inferred from descriptive comparisons of the FAF patterns seen in diseased eyes against those seen in normal eyes rather than by involving quantitative comparisons against a gold standard test. The use of FAF imaging for monitoring the extent of geographic atrophy,^{4,6–12} and for monitoring the presence and extent of the 'autofluorescent ring' of retinitis pigmentosa,^{7,23–30} are examples of where there is expert consensus that characteristic patterns of FAF are associated with disease progression, yet this has not been established by quantitative comparisons of imaging methods (other than correlations between FAF intensity and the spatial distribution of retinal pathology). Currently, there is no universally accepted standard approach for quantifying FAF,^{4,72} although image grading software has been developed that can quantify the local or general background intensity of the greyscale FAF image obtained from cSLO, so as to identify which pixels could be classified as indicating hypo- or hyper-autofluorescence.⁷² For FAF imaging to be used in clinical ophthalmology in a consistent way, it would be necessary to determine and agree on thresholds for increased or decreased FAF (ie, pixel intensity) that can be reliably considered as abnormal for different retinal conditions.

The position of FAF imaging in the care pathway

A new test such as FAF imaging could have one of three main roles: triage, add-on, or replacement.⁹⁹ In 5 of the total 12 studies that were included when considering both the original review and the review update, the aim was primarily to explore relationships between FAF and retinal morphological characteristics rather than to compare the accuracy of imaging tests.^{70–72,79,80} One study specifically asked whether FAF imaging could be an ancillary test, for detecting cystoid macular oedema,³⁹ while one study specifically compared FAF imaging as an index test against fluorescein angiography as the reference standard for diagnosing cystoid macular oedema,⁴¹ although the authors did not explicitly discuss whether FAF imaging could replace angiography. The authors of several studies suggested that FAF imaging could assist diagnosis of retinal conditions as one of the several imaging modalities (ie, as an additional rather than a replacement test), for reticular pseudodrusen in AMD,^{73,80,91} diabetic macular oedema,^{48,74} hydroxychloroquine-induced retinal toxicity,⁷⁹ and retinal lacquer cracks.⁸²

When interpreting the studies on FAF imaging, it is important to keep in mind that finding out whether a test can serve its role is not exclusively based on sensitivity and specificity of the test alone, but also on how the accuracy of the existing testing pathway is changed by the test.⁹⁹ Although most of the studies included in our

systematic review suggest that FAF imaging has potential as an add-on test, alongside other retinal imaging methods (eg, optical coherence tomography, plus other tests such as angiography or fundus photography as needed), none of the studies calculated the diagnostic accuracy of a multiple-test strategy.

Could FAF imaging replace fluorescein or indocyanine green angiography?

Given that FAF imaging is non-invasive, there might be benefits to both patients and the health service if FAF imaging could, in some cases, replace retinal angiography, which is the most frequently used invasive retinal imaging test. Retinal angiography carries a risk of complications, including adverse hypersensitivity reactions to dye, ranging from mild nausea or hives to (less commonly) anaphylaxis and death. It is also contradicted in pregnancy, and requires specialist clinical facilities, including a sterile clinical environment and staff qualified in venous access.³⁹ The extent to which FAF imaging might replace retinal angiography would likely depend on the pathological process of the retinal disease. For example, angiography is valuable in AMD and macular oedemas for detecting fluid perfusion and leakage that might not be detectable using FAF imaging. However, of the four studies that employed angiography as a reference standard, none tested whether FAF imaging could replace fluorescein angiography^{39,41,70} or indocyanine green angiography.⁷⁹ These studies also did not report patients' perceptions of the test procedures or whether or not the angiography reference standard was associated with any adverse events. Further evidence would be helpful to clarify the magnitude of benefits or disadvantages to patients and health services of any switch from fluorescein angiography to FAF imaging.

Quality assurance of FAF imaging

A problem in clinical ophthalmology is that formal training and clinical guidelines regarding the use of new imaging techniques in diagnosing and monitoring various ocular conditions is currently lacking.¹⁰⁰ It is important that clinicians (as well as researchers) using cSLO for FAF imaging are aware of the limitations of the method, and receive adequate training in how to operate the equipment in a standardised way and in how to interpret the resulting FAF images. Despite subjectivity in the interpretation of FAF images,⁷⁹ our systematic review indicates that these quality assurance steps are rarely considered in research studies, so the reliability of FAF image interpretation when compared both between image graders and for the same grader at different times is largely unknown.

Limitations of the review

Our systematic review has three potential limitations. First, for practical reasons, we limited searches to studies published in English. We cannot rule out that relevant studies might have been published in other languages. Second, we did not contact authors to clarify any missing or unclear information in the included studies. However, we believe it unlikely that this would have affected our conclusions since major limitations (eg, inappropriate reference standards and/or lack of clinical relevance) were evident in most of the studies based on the peer-reviewed information that was available. Third, to keep the review manageable, we excluded retinal conditions that resulted from malignancy, trauma, or were secondary to other ocular diseases (such as glaucoma). The scope of our conclusions therefore excludes studies on these particular conditions.

Conclusion and recommendations

In conclusion, the scientific literature identified in our searches demonstrates that there is considerable interest in the use of FAF imaging for characterising and describing retinal conditions; however, only a small proportion of the literature met our inclusion criteria, indicating a lack of quantitative assessments of the accuracy of the method for diagnosis or monitoring. We have identified a need for prospective quantitative research into the accuracy of FAF imaging, particularly in the inherited retinal dystrophies, geographic atrophy, and central serous chorioretinopathy; these are important retinal conditions for which we identified no quantitative test accuracy evidence. Our systematic review also highlights that there is a need for improved reporting of research on FAF imaging. In particular, future research studies should report:

- which information, if any, alongside the results of FAF imaging tests is used to inform diagnostic or other clinical decisions, so as to clarify whether test results are being interpreted in the research studies in the same way that they would be in clinical practice;
- the training and experience of image graders;
- inter-grader and intra-grader agreement in classification of FAF images;
- any resources associated with the acquisition, processing, quality assurance, and interpretation of FAF images—this would help to clarify the pros and cons of FAF imaging relative to other imaging modalities (and would also assist any evaluation of the cost-effectiveness of FAF imaging that are conducted).

Conflict of interest

The authors declare no conflict of interest.

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