Looking Ahead: Visual and Anatomical Endpoints in Future Trials of Diabetic Macular Ischemia

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Diabetic macular ischemia · Endpoints · Optical coherence tomography · Microperimetry

Abstract
Diabetic macular ischemia (DMI) is a common complication of diabetic retinopathy that can lead to progressive and irreversible visual loss. Despite substantial clinical burden, there are no treatments for DMI, no validated clinical trial endpoints, and few clinical trials focusing on DMI. Therefore, generating consensus on validated endpoints that can be used in DMI for the development of effective interventions is vital. In this review, we discuss potential endpoints appropriate for use in clinical trials of DMI, and consider the data required to establish acceptable and meaningful endpoints. A combination of anatomical, functional, and patient-reported outcome measures will provide the most complete picture of changes that occur during the progression of DMI. Potential endpoint measures include change in size of the foveal avascular zone measured by optical coherence tomography angiography and change over time in best-corrected visual acuity. However, these endpoints must be supported by further research. We also recommend studies to investigate the natural history and progression of DMI. In addition to improving understanding of how patient demographics and comorbidities such as diabetic macular edema affect clinical trial endpoints, these studies would help to build the consensus definition of DMI that is currently missing from clinical practice and research.

Introduction
Diabetic macular ischemia (DMI) is a complication of diabetic retinopathy (DR) and is associated with a poor prognosis; in some patients, DMI results in severe loss of vision. DMI is a causative and potentially dominant factor for progressive visual impairment experienced by patients with DR, and greater DMI severity (graded by the Early Treatment Diabetic Retinopathy Study [ETDRS] protocol) is associated with a greater degree of visual loss [1, 2]. However, the ETDRS used fluorescein angiograms as the diagnostic methodology, which are unable to image the deeper capillary networks of the eye [3]. Recent advances in retinal imaging that improve visualization of the different retinal plexi (optical coherence tomography angiography,...
phy [OCTA]) have renewed interest in better understanding DMI. Nonetheless, at present there is no consensus regarding the definition, classification, diagnostic criteria, or even the optimal testing modalities for DMI.

Despite the limited literature available on DMI and a lack of consensus on its definition, several features are typically associated with its presence as part of DR. Changes occur in the retinal basement membrane, followed by pericyte loss, resulting in breakdown of the blood-retinal barrier. The loss of macular capillary vasculature may create larger intercapillary spaces, increasing oxygen diffusion time and eventually causing chronic hypoxia [2, 4, 5]. Leukostasis due to adhesion of leukocytes to abnormal endothelial cells may also contribute to capillary occlusion [6]. Fluid leakage from the diseased capillary bed may result in cystoid spaces in the retina, especially in the outer plexiform layer [7]. Ultimately, these events can lead to ischemia and edema in the retina [8, 9].

The degree of capillary bed ischemia required to cause loss of vision is unknown, but it may vary for different visual functions.

Although the precise relationship between retinal vasculature in different layers of the retina is unclear, DMI is associated with decreased retinal vessel density and/or non-perfusion of the macular superficial capillary plexus (SCP), deep capillary plexus (DCP), and choriocapillaris [10–14]. The capillary plexi form part of a dense vascular system that supply oxygen to the macula, with the SCP located in the retinal nerve fiber layer, and the DCP located at the outer border of the inner nuclear layer [15]. Damage to the retinal vasculature affects local oxygenation levels, which are vital for effective functioning of the macula; visual loss and macular photoreceptor disruption have been linked to non-perfusion of the DCP, as well as a decrease in SCP and DCP vessel density [15–17].

The degree of visual loss associated with DMI may depend on the relative level of ischemia between the SCP and DCP, with DCP ischemia causing more serious problems [15]. This is because the DCP plays a role in supplying blood to photoreceptors, which have a high oxygen demand [15, 18]. It may be surmised that there could be different phenotypes of DMI based on the relative abnormalities of the SCP and DCP. However, in our experience, identifying phenotypes of disease using images of the plexus is challenging due to the limited resolution of current imaging methods and/or imaging software.

DMI is also associated with enlargement and disruption of the foveal avascular zone (FAZ), the health of which is integral to maintaining normal visual acuity [19–21]. That said, there is some disconnect between an enlarged and disrupted FAZ and neurosensory changes: not all enlarged or disrupted FAZ result in visual loss [22]. Loss of the retinal neurosensory layer is most likely a sequelae of hypoxia caused by ischemia [2]. Ischemia has a notable impact on the integrity and sensitivity of photoreceptors [13], and thinning of the retinal nerve fiber layer is also seen in patients with DMI [4]. Damage to the neurosensory retina and the retinal blood vessels from DMI forms a vicious circle: damage to the blood vessels of the retina leads to retinal neurosensory damage, and neurosensory damage promotes retinal capillary damage [2]. Furthermore, changes in neural activity within the retina induce changes in vascular flow and density, reinforcing the link between vasculature and neural tissue damage [23].

While there is limited research on the prevalence of DMI, it has been found in 46–77% of patients with DR, with higher prevalence in patients with more severe DR [1]. DMI is frequently associated with other complications of DR, most notably diabetic macular edema (DME). Retinal hypoxia caused by DMI results in upregulation of vascular endothelial growth factor, which can lead to edema [1, 4, 24]. Chronic edema tends to recur in the same locations [25], which may represent regions of focal capillary loss at the macula [25, 26]. However, the relationship between DMI and DME is unclear; some studies show that loss of visual acuity in DME may correlate with severity of macular ischemia in the eye, while other studies show no relationship [1, 27, 28].

As DMI is an important feature of DR progression and prognosis, establishing an accurate grading system is of great importance. Currently, there is no independent published system for grading DMI severity; however, DR is graded using the ETDRS grading system, and in some studies DMI has been graded as ETDRS-DMI using this system [1, 10, 29]. The ETDRS-DMI qualitatively grades DMI severity based on FAZ size using images acquired with fluorescein angiography (FA) [10]. However, some have questioned whether the ETDRS grading system remains relevant with the advent of higher-resolution imaging systems [30]. FA imaging is a 2D modality, and therefore cannot visualize the different retinal plexi, and leakage of fluorescein from the abnormal capillary bed can obscure the true FAZ boundary [31, 32].

**Current Guidelines and Treatment**

In addition to the lack of consensus on the definition of DMI, current guidelines for DR only recommend identification of DMI, rather than suggesting specific man-
Visual and Anatomical Endpoints for Diabetic Macular Ischemia

Management options [33, 34]. Primarily, 2 imaging methods are used to detect DMI by assessing associated anatomical changes in the retina: optical coherence tomography (OCT) and FA [33, 34]. Historically, FA has been the primary diagnostic method for assessing macular disease [11]. Despite being the current gold standard, FA is invasive, costly, and time-consuming, taking roughly 10 min for framing acquisition; it also requires venipuncture [11, 35]. Furthermore, FA is associated with potentially severe medical complications for the patient [36]. OCT imaging only provides indirect evidence of DMI, but more recently, OCTA has been used to image the retinal microvasculature. Unlike FA, OCTA is a non-invasive method that can visualize blood vessels in 3D, thus showing retinal ischemia in greater detail [11, 34, 35]. OCTA devices use Fourier-domain and swept-source implementation to acquire high-resolution images in just 2–3 s [37, 38]. As an evolving technology, imaging artifacts remain a challenge when interpreting changes using OCTA [39–41]. However, software and hardware improvements are expected to resolve these challenges.

Despite its clinical burden, there are currently no approved therapies for the management of DMI [1, 2, 33, 34]. Furthermore, DMI may have a negative impact on the efficacy of treatment for other DR-associated complications, such as anti-vascular endothelial growth factor treatment for DME [42], highlighting the need for DMI-specific therapies.

Generating consensus on validated anatomical and/or visual endpoints that can be used in DMI is vital for the development of effective interventions [43].

Appropriate Endpoints for Use in Clinical Trials of DMI

Given the lack of literature available on DMI, identifying appropriate endpoints is challenging: very few, if any, clinical trials focus explicitly on DMI treatment. However, endpoints used in trials for other ocular diseases that seek to assess similar symptoms may be appropriate for use in DMI. Geographic atrophy (GA), an advanced form of age-related macular degeneration (AMD), may be a relevant comparator. Although DMI and GA are very different (affecting the retinal plexi and choriocapillaris, respectively), it can be argued that the signs of DMI follow a similar “patchy” visual loss pattern as observed in GA [44–46]. Therefore, endpoints that have been used in clinical trials of GA may be of interest, including those previously explored in the observational MACUSTAR study, including microperimetry, low luminance acuity, and contrast sensitivity [47]. Similarly, endpoints discussed in the National Eye Institute (NEI)/US Food and Drug Administration (FDA) workshops for DR and AMD, such as the ETDRS letter chart and visual field modelling and analysis (VFMA) with microperimetry, might be of interest [43, 48].

Visual Function Endpoints

Best-Corrected Visual Acuity

Change in best-corrected visual acuity (BCVA) is the most commonly used endpoint to assess visual function in eye disease [13]. Some patients with DMI are reported to have BCVA as low as Snellen 20/400 [5]. BCVA is determined by the patient reading a letter chart at a distance; the letters decrease in size line by line [49]. The Snellen letter chart is a widely used method in clinical practice for the assessment of visual acuity [49]. However, due to a number of shortcomings, including poor reliability and repeatability, other measures of acuity such as the logMAR-based ETDRS letter chart are preferable for clinical research [49, 50]; for US FDA registration trials, the ETDRS chart is required to assess visual outcomes [49]. The proportion of patients with a change of at least 15 letters in ETDRS BCVA relative to baseline is generally an accepted endpoint in clinical trials of retinal disease [48, 51].

Assessment of BCVA has been used as the primary endpoint in a number of clinical trials, including those assessing aflibercept, bevacizumab, and ranibizumab in DR [52–54] (Table 1). However, European regulators have noted the shortfalls of BCVA as a functional endpoint, highlighting the need for novel functional endpoints [48]. Measurement of BCVA with a letter chart often provides insufficient information on the actual function of the retina due to foveal sparing and parafoveal scotoma in GA [55, 56]. Despite its prevalence as an endpoint in retinal diseases, BCVA may not accurately capture the problems faced by those with DMI [1]. The mean change in number of letters from baseline at a particular time point, such as 12 months, is another possible endpoint; a change of 4–6 letters at 12 months may be clinically meaningful. However, this has not yet been used as an endpoint.

Low Luminance Vision Testing

Low luminance vision testing is designed to replicate the real-world problem of reduced vision in low illumina-
tion. If DMI predominantly affects the DCP, it could reduce blood supply to the photoreceptors with resultant decrease in low luminance acuity. Many patients with early-stage DR have impaired rod photoreceptor function and impaired dark-adapted visual sensitivity [57]. As such, low-luminance vision testing may provide a more DMI-relevant measure of visual function than BCVA. Low luminance vision can be measured by placing a neutral density filter in front of the study eye and asking the participant to read an illuminated letter chart, or by inserting a mesopic filter in front of the letter chart [58, 59]. Low luminance deficit (the difference between normal and low luminance measures) is predictive of future visual acuity loss in GA [60]. However, it is important to note that low luminance visual acuity primarily tests foveal function and therefore may not detect changes as a result of DMI.

### Contrast Sensitivity

Measures of contrast sensitivity are often used to assess visual function and may be better than measures of visual acuity at tracking the progressive loss of vision associated with eye diseases [61, 62]. Contrast sensitivity testing evolved because it was shown that those with AMD had poor contrast discrimination. Similarly, DR is associated with a loss of contrast sensitivity [63].

The Pelli-Robson test for contrast sensitivity comprises letters arranged into triplets, with 2 triplets per line; the intensity of the letter contrast decreases from one triplet to the next [64]. The Pelli-Robson chart is considered the gold-standard contrast sensitivity measurement but can be difficult to set up accurately and illuminate evenly, as well as to replicate at each visit [64]. Additionally, the range of measurements is small, making it difficult to assess subtle differences between patients.

## Table 1. Visual function endpoints

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Test</th>
<th>Indication</th>
<th>Clinical trials</th>
<th>Current data on DMI</th>
<th>Suitable for DMI?</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA</td>
<td>Mean change in BCVA</td>
<td>DR, DME, AMD, IRD</td>
<td>NCT03904056 (DR) NCT03246152 (DME) NCT02432547 (DR) NCT03349801 (AMD) NCT00999609 (IRD) NCT03714308* (AMD) NCT00327470 (AMD)</td>
<td>Patients with moderate-to-severe DMI may have vision ranging from 20/63 to 20/80 [1], but some patients have been reported to reach 20/400 [5]</td>
<td>Yes</td>
</tr>
<tr>
<td>Low luminance visual acuity</td>
<td>Mean change from baseline</td>
<td>AMD</td>
<td>NCT03349801</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Contrast sensitivity</td>
<td>Change in visual performance</td>
<td>RA, AMD</td>
<td>NCT02147171 (RA) NCT00327470 (AMD)</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Microperimetry</td>
<td>Scotopic/mesopic sensitivity</td>
<td>AMD</td>
<td>NCT03349801</td>
<td>A value of 18 dB is considered to be typical retinal sensitivity [13]; one clinical study reported that retinal sensitivity ranges from 10.7 to 12.8 dB in patients with DMI [5]</td>
<td>Yes</td>
</tr>
<tr>
<td>mfERG</td>
<td>Change from baseline</td>
<td>GA</td>
<td>NCT03919019 NCT01258335</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Dark adaptometry</td>
<td>Change in visual performance</td>
<td>RA</td>
<td>NCT02147171</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Reading speed</td>
<td>Vision impairment in low luminance, mean change in reading speed</td>
<td>AMD</td>
<td>NCT03349801 NCT00327470</td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Trial ongoing. AMD, age-related macular degeneration; BCVA, best-corrected visual acuity; DME, diabetic macular edema; DMI, diabetic macular ischemia; DR, diabetic retinopathy; IRD, inherited retinal dystrophy; mfERG, multifocal electroretinography; RA, retinal aging.
Later, the Mars Letter Contrast Sensitivity Test was developed to address the shortcomings of the Pelli-Robson chart, such as the viewing distance and large, impractical chart size [65]. Unlike the Pelli-Robson chart, the intensity of the letter contrast decreases gradually over the course of the Mars chart, with the first letter displaying the highest contrast and the last letter displaying the lowest [64, 66]. Notably, patients with low vision tend to have better repeatability when tested with the Mars chart versus the Pelli-Robson chart [64].

More recently, the Ora variable contrast flicker test (Ora-VCF™) has been developed [67]. A diffuse light source is used to bleach most of the patient’s cone photopigment, after which macular recovery is tracked using a variable contrast flickering stimulus [67]. The Ora-VCF has previously identified significant differences in visual performance between patients with and without GA, which were not picked up by the Pelli-Robson chart [68].

However, contrast sensitivity may be a less clinically meaningful measure of vision for patients and may not have sufficient sensitivity to detect changes in DMI. Furthermore, in our experience, it can be challenging to replicate the lighting conditions during each session, rendering accurate tracking of improvements difficult.

**Microperimetry**

Another possible endpoint for measuring visual function in DMI is microperimetry. Microperimetry combines fundus imaging with functional measures to detect retinal sensitivity by measuring the minimum light intensity that patients can perceive when spots of light stimulate specific areas of the retina and has the potential to map structure to function in the retina [2, 5, 13, 69, 70]. High-resolution maps of retinal sensitivity can be created with the use of VFMA, which allow for topographic correlation with other imaging modalities [71].

Microperimetry devices, such as Macular Integrity Assessment (MAIA), are US FDA approved [72], and microperimetry using the MAIA and Nidek systems can be adjusted for patients with poor vision by using an enhanced fixation light. Microperimetry can track the fundus even in patients who have unsteady or non-foveal fixation, which is vital for accurate measurements in patients with visual loss [69]. Although scotopic microperimetry can be quite time-consuming [73], mesopic microperimetry may be a reliable and quicker alternative that requires minimal dark adaptation [74, 75].

However, the availability of multiple microperimetry devices may make standardizing measurements between clinical centers a challenge. In addition, microperimetry uses complex machinery and requires a trained specialist, which may not be practical in all clinical situations. Despite this, microperimetry remains an attractive endpoint for metrics of retinal sensitivity in DMI, especially if short, custom-made programs can be developed [76].

**Multifocal Electroretinography**

Multifocal electroretinography (mfERG) is one method of recording retinal responses to stimuli and may be appropriate as a measure of retinal function in DMI [2, 77]. During mfERG testing, patients view a fast sequence of images on a monitor while retinal response is recorded [77]. However, one previous study has indicated that mfERG amplitudes may not correlate with ischemic areas of the retina [78]. Furthermore, mfERG is time-consuming and requires a skilled technician. As such, mfERG is unlikely to be an ideal endpoint in clinical trials of DMI.

**Dark Adaptometry**

Similar to the Ora-VCF, dark adaptometry measures the length of time it takes for the retina to regain maximal sensitivity to low amounts of light after it has been exposed to bright light then returned to darkness [79–81]. Dark adaptometry has been used in patients with diabetic retinopathy, showing that rod recovery function degrades earlier than cone function [81]. Time to recovery of baseline visual sensitivity after a period of photostress has also been used as a measure of macular function in elderly subjects (mean age 75 years) [67].

Although dark adaptometry is a quantitative and reproducible measure of photoreceptor function, it is time-consuming (∼30 min per eye) and is unlikely to isolate the impact of DMI on visual function; this means that it is not a particularly promising endpoint for clinical trials of DMI.

**Reading Speed**

Reading speed is a functionally important measure of visual ability from the patient’s perspective. Tests of reading speed, such as MNREAD and International Reading Speed Texts (IReST), may provide practical visual function endpoints that measure the impact of DMI on daily tasks [82–84].

In MNREAD and IReST, patients read through text aloud while being timed by an investigator [82, 83]. MNREAD is a continuous text reading acuity chart; every 3 lines, the size of the text decreases by a certain font point number [83]. Reading acuity, maximum reading speed, and critical print size (smallest print that supports the maximum reading speed) can be determined via MN-
Tests of reading speed provide a functionally relevant endpoint that may be sensitive to local disturbances in vision, such as those caused by DMI. Although there are some shortcomings for use in certain patient populations (e.g., those who are illiterate or for whom an equivalent translation has not been developed) and in the test duration, they are a meaningful measure of improvement from a patient perspective. IReST may represent a more realistic daily reading situation than MNREAD and has the advantage of not being limited to English-speaking participants.

Visual Function Endpoints for DMI

Currently, BCVA is as an established and regulator-approved endpoint; however, it may not have adequate sensitivity to detect change in patients with DMI. As such, including other measures of visual function is important in trials of DMI. Microperimetry measures of retinal sensitivity may be promising as a functional endpoint in DMI and may detect changes that are too subtle to detect using BCVA. Developing a suitable, short, and validated microperimetry protocol for use in patients with DMI will be a vital first step prior to the conduct of any clinical trial. Tests of reading speed and low luminance BCVA may provide insight into how improvements in visual function result in better functional vision for patients with DMI. Furthermore, reading tests may be more sensitive to local visual disturbance resulting from scotoma/FAZ disruption than BCVA. However, DMI is unlikely to cause deep scotomas, and thus reading speed may not reveal any significant changes as a result of treatment over the course of a typical 12-month clinical trial. Crucially, measures of visual function must be correlated with appropriate anatomical measures to provide a complete picture of retinal changes over time.

Anatomical Endpoints

DMI is primarily identified by anatomical hallmarks [34]; it follows that endpoints focusing on changes in retinal anatomy may be useful in clinical trials. FA and OCTA are US FDA-approved methods for acquiring anatomical measurements of the retina. At present, 4 OCTA devices have been approved by the US FDA [38]. Image processing software (e.g., ImageJ [Fiji] and MATLAB) can be used to quantify features seen with OCTA, such as the level of macular non-perfusion [18].

Optical Coherence Tomography Angiography

OCTA is a relatively novel method for assessing visual disease; little is known about which OCTA metric is most likely to be associated with visual or functional loss. Commercial devices that can use wide-field OCTA patterns are now available [86] and may be critical in characterizing the full extent of DMI pathology. As a relatively new technology, it is worth noting that there is no consensus on terminology for OCTA metrics across different retinal diseases; however, there is growing interest in establishing such a consensus nomenclature [87].

A number of issues with OCTA and OCT need to be resolved before their metrics can be incorporated as endpoints in clinical trials. These include the variability of measurements from the multiple instruments available (each with their own segmentation software and associated errors), problematic image artifacts typically caused by patient eye movements, and failure of the operator to focus and center the scan [39–41]. At present, OCTA is more prone to artifacts than OCT [88]; for example, segmentation failure may occur during image analysis, particularly when retinal pathology is present [40]. Furthermore, as OCTA image reconstruction methods are designed to enhance retinal vasculature, they may also enhance background elements that by chance share image features with genuine blood vessels [89], confounding DMI-relevant measures of perfusion. The formation of cataracts and edema in particular can have a substantial effect on OCTA measurements of retinal blood flow [90]. As the technology develops, it will be critical to develop algorithms that can account for the specific pathology of DMI during image segmentation and analysis.

Several OCTA metrics may be appropriate anatomical endpoints for DMI. Change in FAZ size is one potential endpoint because increased FAZ size is associated with DMI [1, 21, 91]. One recent publication has indicated that larger FAZ area as measured by OCTA predicts worse visual outcomes in patients with DR [92]. Change in the FAZ has been used as an endpoint in clinical trials of bevacizumab and pegaptanib in ischemic DME and ischemic maculopathy (Table 2) [53, 93]. Notably, measures of FAZ by OCTA are robust even in the presence of cataracts [90], which is important given that the risk of cataracts and DR increase with age [94, 95]. Measurements of vessel density and/or capillary perfusion may also be insight-
ful endpoints in DMI trials. Although such measures are often collected in current trials, they are not typically assessed as main outcomes, and it remains unclear whether these metrics are predictive of functional loss. Decreased capillary perfusion and vessel density in the SCP, DCP, and choriocapillaris are linked to DMI [10–14]. Capillary perfusion has been used as an endpoint in clinical trials of bevacizumab and the dexamethasone intravitreal implant in DR and ischemic maculopathy (Table 2) [53, 96]. Retinal capillary closure quantified by OCTA has recently been shown to identify and predict the severity progression of DR and may be considered as a proxy measurement of DMI extent [97, 98]. Finally, some evidence suggests that DMI may be a precursor of DME/proliferative diabetic retinopathy (PDR); OCTA metrics of decreased vessel density in the SCP can predict conversion to DME [35], while reduced measures of vessel density in the DCP can predict conversion to PDR [35]. Quantifying treatment-related delay in these conversions using OCTA may be a meaningful endpoint in clinical trials of DMI.

**Optical Coherence Tomography**

In addition to OCTA, OCT metrics could serve as useful endpoints. Retinal thickness has been used to assess disease in patients with DR [52, 93]. A reduction in inner retinal thickness, as measured by OCT, may be an indicator of DMI in early-stage DR [99]. However, evaluating retinal thickness in the context of DMI is challenging because anatomical changes progress slowly [4, 20, 100]. In addition, it should be noted that common DR comorbidities, such as DME, can cause misidentification, segmentation errors, and artifacts in OCT imaging [101], which could interfere with imaging of DMI. This, alongside the slow progression of neurodegeneration, indicates that retinal thickness may not be a reliable anatomical endpoint for patients with DMI. Another possible OCT metric is the detection of change in the ellipsoid zone (EZ). EZ defects or “breaks” are believed to be the product of photoreceptor loss [102], and OCT measurements of EZ defects correlate with changes in retinal sensitivity measured by microperimetry [103, 104]. Rate of change in the EZ area has been used as a primary endpoint in phase 3 clinical trials of macular telangiectasia [105], an ocular disease that can involve ischemia [106]; thus, it may be appropriate as an endpoint in advanced clinical trials of DMI.

**Disorganization of the Retinal Inner Layers**

Disorganization of the retinal inner layers (DRIL) is defined as the inability to identify boundaries between the ganglion cell-inner plexiform layer complex, inner nucle-

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**Table 2. Anatomical endpoints**

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Test</th>
<th>Indication</th>
<th>Clinical trials</th>
<th>Current data on DMI</th>
<th>Suitable for DMI?</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAZ size</td>
<td>Change in FAZ area and/or perimeter by OCTA</td>
<td>iDME, IM</td>
<td>NCT01175070 (iDME) NCT03246152 (IM)</td>
<td>The size of the FAZ can increase by up to 0.94 mm in DMI [2] or by 5–10% of the baseline FAZ area per year [21]</td>
<td>Yes</td>
</tr>
<tr>
<td>Vessel density/perfusion</td>
<td>Capillary perfusion/density by OCTA</td>
<td>RI, DME, DR, IM</td>
<td>NCT04038125 (DME, RI) NCT03246152 (DME, IM)</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Conversion to DME/PDR</td>
<td>OCTA metrics of decreased vessel density in the SCP</td>
<td>None as of yet</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Retinal thinning</td>
<td>Mean change from baseline by OCT</td>
<td>AMD, DME</td>
<td>NCT03349801 (AMD) NCT04163968* (DME) NCT03714306* (AMD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area of ellipsoid zone defects</td>
<td>Rate of change in ellipsoid area defect measured by OCT</td>
<td>MT</td>
<td>NCT03316300</td>
<td>Yes (in phase 3)</td>
<td></td>
</tr>
<tr>
<td>DRIL</td>
<td>Presence</td>
<td>DME</td>
<td>NCT04163968*</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Adaptive optics</td>
<td>High-resolution imaging</td>
<td>AMD, GA</td>
<td>NCT01866371*</td>
<td></td>
<td>No (yes for exploratory PoCP)</td>
</tr>
</tbody>
</table>

* Trial ongoing. AMD, age-related macular degeneration; DME, diabetic macular edema; DR, diabetic retinopathy; DRIL, disorganization of retinal inner layers; EMA, European Medicines Agency; FAZ, foveal avascular zone; iDME, ischemic DME; IM, ischemic maculopathy; GA, geographic atrophy; mfERG, multifocal electroretinography; MT, macular telangiectasia; OCT, optical coherence tomography; OCTA, optical coherence tomography angiography; PDR, proliferative diabetic retinopathy; PoCP, proof of clinical concept; RI, retinal ischemia; SCP, superficial capillaryplexus.
The presence of DRIL may be a proxy for the severity of DMI in the retina and thus could be a useful endpoint. DRIL measurements using spectral-domain OCT have been suggested as potential biomarkers of visual recovery in eyes with center-involved DME [42]. Although DRIL is associated with retinal capillary non-perfusion (i.e., DMI), the absence of DRIL does not rule out the presence of DMI [17, 107]. However, the grading of DRIL may be subjective, limiting its use as a clinical trial endpoint.

**Adaptive Optics**

Adaptive optics (AO) laser scanning ophthalmology is a non-invasive imaging technique that has been used to measure retinal blood flow and velocity in patients with DR [108, 109]. AO can visualize individual rods and cones, enabling detailed assessment of retinal structure [110]. The Imagine Eyes rtx1™ AO retinal camera has been able to identify microscopic hemorrhages, edematous cyst walls, modified arteriolar structure, and microaneurysms in patients with DR [111–113] and those with pre-diabetic conditions [114]. However, at present, there are only 2 commercially available AO machines (Imagine Eyes rtx1 and Boston Micromachines Apaeros™ Retinal Imaging System [115, 116]), which may limit its widespread use in clinical trials. In addition, AO is extremely time-consuming (~1 h), and the currently available software for quantification is only used in research. Although AO may be a promising future endpoint, it is not appropriate in its current form. However, the prevention or reduction of further photoreceptor loss measured by AO could be used as an endpoint to establish efficacy in a proof-of-clinical principle study.

**Anatomical Endpoints for DMI**

At present, OCTA appears to be the most precise, quantifiable, and objective method for detecting anatomical features linked with visual loss and DMI. The image quality and reliable range of metrics OCTA provides will be invaluable in the assessment of outcomes for clinical trials of DMI. Although the most appropriate metric is unknown, potential OCTA endpoints include the size of the FAZ within the SCP or DCP, and measures of vessel density and perfusion. Future hardware and software improvements are expected, as there are several companies developing OCTA devices. In addition, the presence of DRIL and conversion to PDR or DME are worth exploring as endpoints. Furthermore, short-term anatomical endpoints that predict long-term irreversible visual function loss, but can be measured over the time course of a typical clinical trial (analogous to changes in GA lesion size that are a marker of future visual loss [117]), should be considered. Such predictive endpoints could be used to establish a 1- or 2-step progression/regression model of DR/DMI grading; similar systems have been shown to strongly predict PDR progression within DR (p < 0.0001) [118].

**Patient-Reported Outcome Endpoints**

Patient-reported outcomes (PROs) that assess patients’ vision-related quality of life are increasingly used in clinical trials (Table 3). PROs are supported by patient advocacy groups and focus on different aspects of visual loss, from functional effects to emotional impact of visual loss.

**EuroQoL 5 Dimensions**

A range of surveys are available, but the most general and widely used health-related option is the EuroQoL 5 dimensions (EQ-5D). The EQ-5D is a standardized outcome measure across 5 domains (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression), designed for self-completion [119]. Although the EQ-5D captures a wide range of health-related endpoints, it may not be specific enough for patients with DMI.

**25-Item National Eye Institute Visual Function Questionnaire**

A more specific and commonly used survey is the 25-item National Eye Institute Visual Function Questionnaire (NEI-VFQ-25), which assesses the influence of visual impairment on health-related quality of life [120]. Scores are assessed using 25 questions across 3 areas (general health and vision, difficulty with activities, and responses to vision problems). Higher scores in the NEI-VFQ-25 equate to better function and quality of life [121]; an increase in score relative to baseline indicates improvement in the relevant area.

**Impact of Vision Impairment Questionnaire**

The Impact of Vision Impairment Questionnaire (IVI) is a 28-item questionnaire (now available digitally) that measures the impact of vision impairment on vision-related quality of life [122]. It comprises 3 subscales: “reading and accessing information,” “mobility and independence,” and “emotional wellbeing.” A decrease in score relative to baseline indicates improvement in the relevant subscale.
Functional Reading Independence

Finally, the Functional Reading Independence (FRI) Index provides insight into a very common visual task (reading) that may also link with more specific measures of visual function, such as MNREAD. The FRI is a 7-item interview-based questionnaire, focusing on daily reading activities under different circumstances (e.g., books, cheque-writing, food labels, television). A higher score indicates a greater level of independence [123]. The FRI has been used as an outcome measure in a clinical trial of GA [124], which may position it as a useful endpoint in DMI.

PRO Endpoints for DMI

PROs may help to highlight statistically significant results that are also clinically meaningful and to demonstrate non-inferiority for interventions that differ in cost [51, 125]. Generally speaking, PRO questionnaires are easy to administer, providing a low-cost measure of changes in patient quality of life. The use of several DMI-relevant PRO endpoints, such as the FRI and NEI-VFQ-25, may give a broader view of trial outcomes.

Conclusions

DMI is a key risk factor for profound visual loss in patients with DR. Despite its substantial impact on patient quality of life and independence, there are currently no approved treatments for DMI. The paucity of literature and lack of consensus on DMI outline how crucial it is to develop a robust and generally agreed definition and severity grading system that can be used consistently in clinical trials. Future studies on the natural history and progression of DMI will help to delineate its relationship with other DR complications, such as DME, DRIL, and PDR. A thorough understanding of DMI will build understanding of how patient demographics and comorbidities (such as hypertension, age, and cardiovascular problems) can affect clinical trial endpoints. In addition, such research would contribute towards the consensus definition of DMI that is currently lacking in clinical practice and research. Establishing a new classification system for DMI based on functional correlations (e.g., retinal threshold sensitivity using microperimetry), with structural features detected by OCT and OCTA, will be central to moving forward.

Table 4 summarizes the advantages and disadvantages of the endpoints discussed in this review. At present, OCTA is the most precise and quantifiable method for detecting anatomical features linked with visual loss and DMI. Using OCTA to examine longitudinal changes in anatomical features of DMI, such as the FAZ size, will provide greater insight into the general progression and prognosis of the disease. Although there are several technical aspects that must be resolved to yield the most reliable data for clinical trials, including comparability between instruments and studies, and the impact of image artifacts, OCTA is a promising imaging method with a variety of potentially DMI-relevant endpoints. Promising endpoints to predict functional visual outcomes in trials could include the stabilization of FAZ size and SCP/DCP vessel density.

OCTA does not provide any functional information, which limits the conclusions that can be drawn from metrics. Therefore, it makes sense to include several func-

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Test</th>
<th>Indication</th>
<th>Clinical trials</th>
<th>Current data on DMI</th>
<th>Suitable for DMI?</th>
</tr>
</thead>
<tbody>
<tr>
<td>EQ-5D</td>
<td>Change from baseline</td>
<td>AMD</td>
<td>NCT03714308* NCT00327470</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>NEI-VFQ-25</td>
<td>Change from baseline</td>
<td>AMD (GA), AMD</td>
<td>NCT01229215 (GA) NCT03714308* (AMD) NCT00327470 (AMD)</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>IVI</td>
<td>Change from baseline at 1 year</td>
<td>AMD</td>
<td>NCT03046485†</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>FRI index</td>
<td>Not an endpoint but recorded</td>
<td>AMD (GA)</td>
<td>NCT01229215</td>
<td>Maybe</td>
<td></td>
</tr>
</tbody>
</table>

* Trial ongoing, † Trial withdrawn due to meeting primary endpoint early. AMD, age-related macular degeneration; DMI, diabetic macular ischemia; EQ-5D, EuroQoL 5 dimensions; FRI, Functional Reading Independence; GA, geographic atrophy; IVI, intravitreal injection; NEI-VFQ-25, 25-item National Eye Institute Visual Function Questionnaire; PRO, patient-reported outcome.
<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visual function endpoints</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCVA</td>
<td>Mean change in BCVA</td>
<td>Commonly used, regulator-approved, validated in other visual diseases</td>
<td>May not be sensitive to changes in DMI, may not detect change over short (e.g., 12-month) trial periods</td>
</tr>
<tr>
<td>Low luminance visual acuity</td>
<td>Mean change from baseline and difference between normal and low luminance (low luminance deficit)</td>
<td>Low luminance vision may be impaired in patients with early DR</td>
<td>Primarily tests foveal function</td>
</tr>
<tr>
<td>Contrast sensitivity</td>
<td>Change in visual performance</td>
<td>May be suitable for tracking progressive loss of vision</td>
<td>Measurement range is small, some tests may be difficult to set up, may be a less clinically meaningful measure of vision, may not be sensitive enough to detect changes from DMI</td>
</tr>
<tr>
<td>Micropertimetry</td>
<td>Scotopic/mesopic sensitivity</td>
<td>High resolution and sensitivity, can be mapped to other imaging modalities, can be adjusted for use with patients who have poor vision or fixation</td>
<td>Scotopic micropertimetry is time-consuming, requires expertise to administer, and can be tiring for the patient</td>
</tr>
<tr>
<td>mfERG</td>
<td>Change from baseline</td>
<td>Provides a measure of retinal function</td>
<td>mfERG may not correlate with ischemic areas of the retina, is time-consuming, and requires a skilled technician</td>
</tr>
<tr>
<td>Disk adaptometry</td>
<td>Change in visual performance</td>
<td>Has been used in DR populations previously</td>
<td>Time-consuming and unlikely to isolate the impact of DMI on vision</td>
</tr>
<tr>
<td>Reading speed</td>
<td>Mean change in reading speed</td>
<td>Functionally important measure from a patient’s perspective, sensitive to local disturbances in vision</td>
<td>Cannot be used in illiterate populations, unlikely to detect change over the course of a 12-month trial</td>
</tr>
<tr>
<td><strong>Anatomical endpoints</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAZ size</td>
<td>Change in FAZ area and/or perimeter by OCTA</td>
<td>Increased FAZ is associated with DMI, has been used in clinical trials, robust even in the presence of cataracts, perimeter irregularities can be seen as DR progresses</td>
<td>FAZ area is very variable even in unaffected populations</td>
</tr>
<tr>
<td>Vessel density/perfusion</td>
<td>Capillary perfusion/density by OCTA</td>
<td>Reduced vessel density and perfusion are associated with DML has been used in clinical trials</td>
<td>Unclear which metrics are predictive of visual loss</td>
</tr>
<tr>
<td>OCTA metrics of decreased vessel density in the SCP</td>
<td>OCTA metrics of decreased vessel density in the SCP</td>
<td>Some evidence suggests DMI may be a precursor to DME/PDR</td>
<td>Limited information available at present, not used in any clinical trials</td>
</tr>
<tr>
<td>Conversion to DME/PDR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinal thinning</td>
<td>Mean change from baseline by OCT</td>
<td>May be an indicator of DML in early-stage DR</td>
<td>Unlikely to detect change over short time frame (e.g., 12 months)</td>
</tr>
<tr>
<td>Area of ellipsoid zone defects</td>
<td>Rate of change in ellipsoid area defect measured by OCT</td>
<td>Has been used as an endpoint in previous trials of ocular disease, is associated with photoreceptor loss, correlates with micropertimetry measures</td>
<td>Has not been studied in DMI</td>
</tr>
<tr>
<td>DRIL</td>
<td>Presence</td>
<td>DRIL may be a proxy for DMI severity</td>
<td>Grading may be subjective, limited information on the relationship between DRIL and DMI</td>
</tr>
<tr>
<td>Adaptive optics</td>
<td>High-resolution imaging</td>
<td>Very high-resolution imaging of retinal structure</td>
<td>Few commercially available machines, very time-consuming, current software only used in research</td>
</tr>
<tr>
<td><strong>PRO endpoints</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EQ-5D</td>
<td>Change from baseline</td>
<td>Widely used measure of health-related outcomes</td>
<td>Not specific to visual diseases, relies on patient report</td>
</tr>
<tr>
<td>NEI-VFQ-25</td>
<td>Change from baseline</td>
<td>Commonly used, vision specific</td>
<td>Relies on patient report</td>
</tr>
<tr>
<td>IVI</td>
<td>Change IVI from baseline at 1 year</td>
<td>Vision specific</td>
<td>Relies on patient report</td>
</tr>
<tr>
<td>FRI index</td>
<td>Not an endpoint, but recorded</td>
<td>Functionally relevant, vision specific, used in clinical trials of GA</td>
<td>Relies on patient report</td>
</tr>
</tbody>
</table>

BCVA, best-corrected visual acuity; DME, diabetic macular edema; DML, diabetic macular ischemia; DR, diabetic retinopathy; EQ-5D, EuroQoL 5 dimensions; FAZ, foveal avascular zone; FRI, functional reading independence; GA, geographic atrophy; IVI, intravitreal injection; mfERG, multifocal electroretinogram; NEI-VFQ-25, 25-item National Eye Institute Visual Function Questionnaire; OCT, optical coherence tomography; OCTA, optical coherence tomography angiography; PDR, proliferative diabetic retinopathy; PRO, patient-reported outcomes; SCP, superficial capillary plexus.
tional measurements as a complement, with a view to correlating functional and anatomical changes. Microperimetry is an attractive functional endpoint for DMI; measurement of change in retinal sensitivity could be a DMI-appropriate endpoint that is sensitive to subtle treatment-related changes, while also having adequate resolution to correlate with high-resolution anatomical imaging methods such as OCTA. In addition, BCVA remains a gold standard, regulator-approved measure of visual function and should be considered as an endpoint in DMI. Tests of reading speed and low-luminance visual acuity could also be considered to provide a greater breadth of information. In addition to anatomical and functional endpoints, PROs such as the NEI-VFQ-25 and IVI may provide useful context for clinical outcomes.

In summary, research confirming suitable endpoints for DMI is needed; identifying a short-term anatomical endpoint that can predict future irreversible visual loss should be a focus of future work. Furthermore, establishing how measures of visual function and PROs map onto the anatomical changes that occur in DMI, and as a result of its treatment, are vital to better understand the disease. The personal views and collated data in this paper are intended as a starting point to guide discussion in the retinal disease community. We recommend that formal study groups comprising clinicians, basic scientists, imaging experts, pharmaceutical and regulatory body representatives be convened to improve understanding of DMI.

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Conflict of Interest Statement

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Author Contributions

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