Ophthalmologica

DOI: 10.1159/000515148
Received: 11/9/2020
Accepted: 2/3/2021
Published(online): 2/10/2021

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ISSN: 0030-3755 (Print), eISSN: 1423-0267 (Online)
https://www.karger.com/OPH
Ophthalmologica

Review Article

Clinical Perspectives and Trends: Microperimetry as a trial endpoint in retinal disease

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Short Title: Microperimetry: trial endpoint in retinal disease

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Number of Tables: 6

Number of Figures: 0

Word count: 12528

Keywords: Microperimetry; Fundus-related perimetry; Fundus-automated perimetry; Endpoints; Outcome measures

Abstract

Endpoint development trials are underway across the spectrum of retinal disease. New validated endpoints are urgently required for the assessment of emerging gene therapies and in preparation for the arrival of novel therapeutics targeting the early stages of common sight-threatening conditions such as age-related macular degeneration and diabetic macular edema. Visual function measures are likely to be key candidates in this search. Over the last two decades, microperimetry has been used extensively to characterize functional vision in a wide range of retinal conditions, often detecting subtle defects in retinal sensitivity that precede visual acuity loss and tracking disease progression over relatively short periods of time. Given these appealing features, microperimetry has already been adopted as an endpoint in interventional studies, including multicenter trials, on a modest scale. A review of its use to date shows a concurrent lack of consensus in test strategy and a wealth of innovative disease and treatment-specific metrics which may show promise as clinical trial endpoints. There are practical considerations to consider in its use, but these have not held back its popularity and it remains a widely used psychophysical test in research. Endpoint development trials will undoubtedly be key in understanding the validity of microperimetry as a clinical trial endpoint, but existing signs are promising.

Introduction

Change in visual function is the US Food and Drug Administrations (FDA) recommended primary endpoint for trials assessing the effect of new therapeutics for ocular conditions.[1] High contrast best corrected visual acuity (BCVA) is the only generally accepted visual function endpoint by regulators and payers. Change in BCVA, specifically a loss or gain of 15 or more Early Treatment Diabetic Retinopathy Study (ETDRS) letters has been successfully adopted in large landmark multi centre clinical trials in ophthalmology over the last decades;[2-5] however its value in assessing functional deficits in early disease and tracking small but important amounts of progression is limited[6-8].

The need for new, validated endpoints in both acquired and inherited retinal disease (IRD) has been widely discussed[9-11], with endpoint development clinical studies currently underway in early and intermediate AMD [12-14] Stargardt disease[8] and Retinitis Pigmentosa.[15] An ideal endpoint would be capable of being easily and frequently measured; be repeatable with having minimal measurement and ascertainment error; be sensitive to change over time and treatment effect; have clinical relevance and be meaningful to patients.[6, 16] Even if these features were confirmed in a laboratory setting, they must also hold across large, international multicenter clinical trial settings if an endpoint is to be truly expedient at assessing novel therapeutics.

Over recent years, there has been a keen uptake in the use of microperimetry (MP) in the research field. Notably, it is listed as a primary or secondary endpoint or keyword in over 150 clinical studies registered online with the United States National Library of Medicine[17]. It has been used to help characterise a wide range of ocular conditions including age-related macular degeneration(AMD)[18]; choroidal neovascularisation (CNV)[19], macular edema arising from diabetes[20] or uveitis[21]; central serous chorioretinopathy (CSCR)[22]; retinal vein occlusion[23]; birdshot chorioretinopathy[24]; macular holes[25]; epiretinal membranes[26] and IRD such as Stargardt disease,[27] choroideraemia,[28-30] juvenile X-linked retinoschisis[31] and *RPGR* associated X-linked Retinitis Pigmentosa.[32] To a lesser extent, MP has already been adopted as an endpoint in interventional retinal disease clinical trials. Given the continued interest in its potential, we sought to characterize this existing uptake, identifying current trends in its use and elucidating potential future directions in endpoint development and validation.

Following a technical review of commercially available microperimeters and their specifications, summarized in Table 1, this manuscript discusses MP as an endpoint in retinal disease clinical trials. Specifically, we review the use of retinal sensitivity measures as primary, secondary or exploratory endpoints in interventional trials. To contextualize the review findings, we also discuss research with the potential to inform MP endpoint development such as natural history studies and studies discussing novel MP metrics. Though fixation stability metrics are provided by microperimeters, given the significant over representation of retinal sensitivity in the literature and breadth of the subject matter, fixation is not explicitly addressed but will be briefly discussed for context.

Main Text

Introduction to Microperimetry

MP is a psychophysical method which probes retinal sensitivity, specifically across the macula. MP is somewhat of a misnomer as the stimulus size (Goldmann size I to V) is comparable to that used in standard automated perimetry (SAP) and the retinal area covered is up to 30^0 (degrees) from the fovea.[33] Alternative terms considered more accurate include 'fundus-guided perimetry' or 'fundus-related perimetry', but the original term has persisted since its first use in the literature in 1990, and thus MP will be used herein.[34]

MP is distinguished from conventional visual fields by its ability to display and track a live fundus image during an exam, whilst adjusting for fixational eye movements. This provides assurance that threshold sensitivity values correspond to specific retinal locations. Diseases affecting the macula can result in unstable and/or eccentric fixation, making MP an attractive tool in their assessment.[35] Additionally, characteristics of fixation may change during disease progression and MP devices are able to quantify and track these changes.

The SLO-101 (Rodenstock, Munich, Germany) was the first commercially available device with MP capabilities. Somewhat rudimentary by today's standards, it lacked automation and repeat assessment of the same retinal locations was not easy to achieve. Additionally, testing was arduous and the device was expensive to maintain. Thus, its use did not become widespread outside academic institutions and eventually it was taken off the market.

Nidek MP-1

The lack of automation was first addressed in 2002 with the arrival of the Nidek Microperimeter-1 (MP-1; Nidek Technologies, Padova, Italy), the first commercially available MP device with an eye tracker. Using a baseline fundus image, high contrast landmarks are manually selected and act as a reference, enabling eye tracking technology to detect changes in eye position every 40 milliseconds [frequency 25Hertz (Hz)]. Requiring a minimum pupil of ~4mm, pupil mydriasis is often required.[36]

With a dynamic range of 0 – 20 decibels (dB), stimuli are projected via a liquid crystal display (LCD) [background luminance of 1.27 candelas per meter squared (cd/m^2)] according to either a standard (macular or peripapillary) or customized grid. Additional test points can be added to a default grid or created *de novo* anywhere across the central 40^0 by way of an onboard 'pattern editor'. Fixation target characteristics are also customizable and stimuli can be presented in Goldmann sizes I to V in white or red.

A black and white infra-red fundus image is viewable during testing. A high-quality color image can be captured at the end of testing, onto which the sensitivity map can be superimposed. Retinal sensitivity maps can also be superimposed on imported images, including fundus autofluorescence (FAF), fluorescein and indocyanine green angiography, thus allowing retinal sensitivity values to be directly associated with retinal lesions. Furthermore, it is possible to import a Heidelberg optical coherence tomography (OCT) line scan prior to testing, to help identify the anatomical fovea. This feature may be particularly desirable in patients with geographic atrophy (GA) where the fovea may otherwise not be readily identifiable.

According to the literature, the MP-1 has the largest scale normative data available for mesopic testing in a microperimeter, incorporating data from 190 healthy subjects between the ages of 20 to 75 years old.[37] This allows generation of a 'local defect map' which presents the differences between obtained sensitivity and age-matched normal values, provided testing utilizes Goldmann III stimuli within a 20° diameter circle centered on the fovea. The MP-1 is also the only microperimeter to offer kinetic testing.

Nidek MP-3

Nidek released the MP-3 in 2014 with a larger dynamic range of 0 - 34dB for light-adapted testing with the option of two background luminances: 1.27 cd/m^2 or 10 cd/m^2. Later in 2018, the MP-3S was introduced, offering scotopic testing facilitated by a background luminance of 0.00095cd/m^2 and scotopic dynamic range of 0 - 24dB. MP-3S is the only microperimeter that can perform scotopic, mesopic and photopic testing.

Compared to the MP-1, MP-3 has improved eye tracking technology, tracking at 30Hz and landmark identification is no longer required. Grid and fixation target customization options have been retained and the same minimum pupil size and dilation recommendations apply. Having no normative database, the MP-3 cannot produce local defect maps and currently does not allow the import of OCT or other images. As kinetic testing was not widely adopted, this feature has been removed. If desired, the option to simulate MP-1 mesopic testing conditions and scale can be selected with the MP-3. This facilitates the continued longitudinal follow up for patients who have had previous mesopic testing on MP-1. This is not possible for scotopic testing given differences in scotopic background luminance.

Optos OCT/SLO

In 2006, the spectral OCT/SLO (OPKO/OTI, Miami, USA), was launched, later renamed the Optos OCT/SLO (Optos, Dunfermline, Scotland, U.K.) when the technology was acquired by Optos in 2012. The OCT/SLO combines spectral-domain optical coherence tomography (SD-OCT) with MP, thus allowing topographical alignment of retinal thickness and light sensitivity measurements, the only device to do so. *En-face* confocal retinal images acquired by scanning laser ophthalmoscope (SLO) are simultaneously acquired alongside cross-sectional SD-OCT retinal images. Its dynamic range matches the MP-1 and many testing parameters are customisable, including grid pattern, stimuli duration, shape and color. Though still in circulation, the OCT/SLO is no longer manufactured.

MAIA

The Macular Integrity Assessment (MAIA, CenterVue, Padova, Italy) microperimeter has been available since 2009. It utilises a near-infrared line confocal SLO for fundus imaging and a light emitting diode (LED) stimulus projector focused on the retina. Instead of using reference landmarks, the MAIA eye tracker registers the entire fundus image, tracking each pixel at 25Hz. With a background luminance of 1.27 cd/m^2, it tests function in the mesopic range and has a dynamic range of 0 – 36 dB, comparing favourably to other devices. A newer version of the device, S-MAIA is able to perform scotopic testing in addition (background luminance of < 0.0001 cd/m^2).

One notable advantage of this device is it requires only a 2.5mm minimum pupil size, often negating the need for pupil dilation. The MAIA provides a bank of standard grids and fixation targets of circles and crosses. Though customized grids can be imported to the MAIA, they must be programmed in an XML (Extensible Markup Language) file and uploaded to the device.

An age-matched normative database for subjects aged 20 to 80 years old, compiled by the manufacturer, informed their development of a Macular Integrity Index. This index is provided in the results under limited circumstances (i.e. for mesopic testing with 4-2 staircase strategy and with a standard grid of 10° diameter containing 37 radially-oriented points centered on the fovea). The Macular Integrity Index categorizes the retinal sensitivity test results into one of three groups: normal, suspect or abnormal. A numerical summary value is also provided, with larger values representing higher likelihood that test findings are abnormal. This is not indicative of disease severity and is distinct from dB sensitivity values.

Compass

The Compass (CenterVue) was released in 2015. Tailored specifically for use in glaucoma, it shares the luminous parameters and sensitivity scales of SAP. With a 60° field of view, it offers 10-2, 24-2 and 30-2 threshold testing, but customized grids are not available. A minimum 3mm pupil is required, obviating the need for pharmacological mydriasis.

Using confocal SLO technology for tracking, it is able to generate true color confocal images as well as red-free images of the optic nerve head. Two threshold strategies available; 4-2 staircase and Zippy Estimation by Sequential Testing (ZEST), the latter being an established adaptive Bayesian algorithm that aims to shorten testing time, like the Swedish Interactive Thresholding Algorithm (SITA) algorithms of SAP. Normative data is incorporated in its software, thus allowing typical SAP measures such as mean deviation and pattern standard deviation plus false negative and false positive reliability indices.

Common to all MP devices discussed so far, Compass results can be viewed as typical topographic retinal sensitivity maps (i.e. superiorly projected stimuli represented on superior retina), but results are also shown as conventional visual field maps, whereby stimuli are displayed according to their location in visual field space (i.e. superiorly projected stimuli represented in inferior visual field space).

The influence of SAP on the design of MP devices is evident in the specifications for their light-adapted testing conditions which have been modelled on those used in popular perimeters. Analogous to the Octopus perimeters (Haag-Streit AG, Koeniz, Switzerland), the MP-1, MP-3 and MAIA have a background luminance of 1.27cd/m^2 for mesopic testing. MP-3 and Optos OCT/SLO's background luminance of 10cd/m^2 for photopic testing is the same as Humphrey perimeters (Carl Zeiss Meditec, Jena, Germany).[38] Key features of all commercially available devices discussed are summarized in Table 1.

Scotopic testing in Microperimetry

In light conditions, MP primarily assesses the function of cone photoreceptors (photopic) or a mixture of cone and rod function (mesopic), as determined by the luminance of the testing conditions. Impaired rod function is known to occur in a range of retinal conditions including AMD[39], retinal telangiectasia[40], CSCR [41], congenital stationary night blindness[42] and rod-cone dystrophy.[43] Affected patients find dimly lit and low contrast conditions challenging.

Isolation of photoreceptor activity is warranted to determine the impact of interventions targeting a particular photoreceptor type. Rod activity may be assessed by scotopic electrophysiological tests[44], dark-adapted perimetry[45, 46] and indices of dark adaption such as the rod-intercept time[47]. Scotopic MP is a welcome addition to the range of clinical tests available. In the case of AMD, it has provided functional evidence of early impaired rod function, confirming what was previously hypothesized from histological analysis.[48, 49]

The scotopic capability of the MP-1S was modelled on a prototype developed by Crossland et. al. Scotopic spectral sensitivity is maximal for light of wavelength 498 - 505nm, which is also the peak absorption wavelength of rhodopsin. By adding a 500nm short pass filter and a 2.0 neutral density (ND) filter to the optical pathway of the MP-1, luminance levels were attenuated to those suitable for scotopic testing.[50] A 500nm short-pass filter blocks wavelengths of light above 500nm. A 2.0 ND filter reduces the intensity by a factor of 100 (i.e. 10^2), however when the two are combined the overall effect is attenuation by a factor of 500. The standard MP-1S model comes with these filters.

A 1.0 ND filter has also been used by researchers with the MP-1S, to attenuate stimuli to the desired level according to an individual's sensitivity values.[51] The purpose of this, as will be discussed later, is to minimize ceiling and/or floor effects so that the attained threshold values mostly fall within the 0 to 20dB dynamic range. However, no correlation between sensitivity values obtained with different ND filters has been validated, thus precluding direct comparison of results from patients tested with different ND filters.[51]

In 2018, scotopic function for the MP-3 (i.e. MP-3S) was introduced with the filters required for scotopic testing in-built. The MP-3S uses a more selective bandpass filter with a peak transmission at 500nm. Background illumination has been reduced further to 0.00095cd/m^2 and this device has a dynamic range of 0 - 24dB. As previously mentioned, this change in background illumination means that longitudinal scotopic testing in patients commenced in the MP-1S cannot cross-over to the MP-3S.

In turn, Centervue released the S-MAIA, whose scotopic feature presents stimuli in two different wavelengths: cyan (505nm) and red (627nm) which help to further isolate photoreceptor activity. This has been validated in a normative study [52]. Each grid location is tested with the cyan stimulus and then red stimulus testing follows thereafter. The S-MAIA generates average threshold sensitivity values for both scotopic cyan and red testing separately, as well as subtracts red values from cyan to give a value for 'cyan-red difference'.

The concept of using two wavelengths of stimuli was first established in modified perimeters to isolate photoreceptor function by exploiting the difference in spectral sensitivity of rods and cones.[53, 54] Generally, under fully dark-adapted conditions in healthy retina outside the rod-free zone, rods are more sensitive than cones at both wavelengths and secondly, the sensitivity to cyan is much higher (around 2 log units) than for red stimuli[53]. The scotopic setting of the S-MAIA has been calibrated according to the CIE 1951 scotopic luminosity function or V'(λ) such that the *radiance* of a sensitivity value for scotopic cyan stimuli is in effect 20dB lower than that for red[54]. Therefore, a cyan-red difference of around 0dB (in areas outside the rod-free zone and in the presence of normal sensitivity values for cyan and red), indicates normal rod function in the S-MAIA[52].

In retinal disease, one may need to exercise caution when interpreting the results of two-wavelength stimuli testing as it cannot be assumed that the sensitivity values obtained are mediated by the same photoreceptor type as for normal eyes (e.g. sensitivity values obtained for cyan stimulus outside the rod free zone which ordinarily would be mediated by rods, may instead be mediated by cones in the presence of rod impairment). Interpretation should involve evaluation of the location of the tested area (given the differing topographical densities of rods and cones according to eccentricity); the cyan and red sensitivity values as compared to normative values but also compared to each other and lastly any device limitations such as floor effects.

To expand on this, in the S-MAIA isolated rod dysfunction (or where rod dysfunction is greater than cone dysfunction) would be reflected in a reduction of cyan sensitivity, while red sensitivity would not be so affected, thus leading to a more negative value for cyan-red difference[55]. However, *severe* rod dysfunction whereby cones mediate sensitivity to both cyan and red stimuli, may not be readily observed due to the floor effects of the device[55]. Isolated cone dysfunction may lead to reduction in scotopic red sensitivity, especially at central retinal locations where the sensitivity values would have been expected to be maximal in a normal eye. Cyan sensitivity would not be as affected.

The first-generation S-MAIA had a dynamic range of 0 - 20dB, however extended minimum and maximum stimulus intensities were introduced in the second-generation device providing an extended dynamic range of 0 - 36dB. Via software upgrade, first-generation data could be automatically converted to equivalent second-generation values. However, <0dB points on the first-generation tests are converted to <10dB as they cannot be further quantified. These changes were accompanied by a change in staircase strategy from 2-1 to 4-2, thus a direct comparison of first- and second-generation S-MAIA data is not, in the very strictest sense, feasible.

Dark Adaptation

To probe scotopic rod-mediated function, a period of dark adaptation (DA) is required prior to testing. The period is based on our understanding of the DA curve, which plots retinal sensitivity over time when a transition is made from light to dark conditions following a period of bleaching or bright light exposure. The initial rapid increase in sensitivity is mediated by cone photoreceptors and after several minutes, reaches a plateau referred to as the cone-rod break. Beyond this period, rods, which are much more operative under scotopic conditions, mediate the further increase in sensitivity, reaching their maximum sensitivity after around 30 to 40 minutes of DA.[56] DA periods for scotopic MP are typically cited as 30 minutes[50, 55, 57-59] although slightly longer DA periods have also been used.[60] Given the additional burden to the patient and the impact on overall examination time, the duration of DA should consider both practical and physiological constraints.

Based on S-MAIA data from normal controls and patients with choroideremia, if starting from normal ambient light conditions, no period of DA is required for mesopic testing provided the eye has relatively preserved cone function.[61] A period of 10 minutes DA is recommended if an eye has had recent exposure to bright light (such as retinal photography or slit lamp biomicroscopy). Therefore, the schedule of tests prior to MP test should be considered.

Scales used in Microperimetry

The scale used in MP follows a similar convention to SAP. To account for the wide range of luminance an eye is responsive to, a logarithmic scale with decibel units is used to measure retinal sensitivity. One decibel unit corresponds to 0.1 log unit change. For example, a dynamic range of 0 - 20dB relates to the differential luminance at the maximum stimulus intensity being 2 log unit (10^2 = 100 times) greater than that at the minimum stimulus intensity. Poorly sensitive areas of retina require brighter stimulus intensities to reach threshold detection. However, as it is intuitive to have low decibel values representing poorer retinal sensitivities, an inverse logarithmic scale is adopted.

Importantly, the decibel range is not an absolute scale and thus the same value in decibels is not the same from one device to another. As such, longitudinal analysis should be performed using the same microperimeter, facilitated by follow-up mode which allows repeat automated testing of the same retinal points regardless of baseline fixation or its subsequent change over time.

The scale for a given device is fixed according to the maximum stimulus intensity available, i.e. 0dB, representing the lowest retinal sensitivity that is quantifiable (i.e. correct response to brightest stimulus intensity is registered). Floor effects refer to the occasions where the observer was not able to detect the brightest stimulus, and therefore the depth of defect cannot be further quantified. These are nominally assigned <0dB or -1dB values. Thus, floor effects, by their nature, may result in underestimation of the defect and represent a heterogeneous group of sensitivity losses.

In determining the impact of an intervention, trialists need to consider where in the dynamic range of the measurement tool, the values derived from the patient population fall. For Phase I/II trials establishing safety, patients with severe disease are often recruited. If at pre-intervention testing patients encounter significant floor effects, the opportunity to track meaningful change post-intervention is reduced; both deterioration and improvement may be masked. For example, patients with neovascular AMD encountered floor effects under mesopic, scotopic cyan and scotopic red testing despite the 0 - 36dB dynamic range of the S-MAIA.[58] Thus, the authors propose patients with mild to moderate disease may make better candidates for interventional studies.

This, however, does not mean the occurrence of floor effects precludes the ability to track meaningful change. Although of limited, strictly quantifiable use, the proportion of points reaching the floor can be tallied up and compared over time or pre- versus post-intervention. Additionally, scotomas by definition are areas of diminished vision surrounded by normal or relatively preserved vision. Therefore, they will commonly, and unavoidably, consist of values which approach or reach the floor. In fact, research groups have defined scotoma-related outcome measures according to type (absolute or relative) and size. That said, static testing may be inferior to kinetic testing when assessing size and borders of scotoma, as the latter technique is not constrained by set spacing intervals between points of a grid.[62]

Comparisons across MP devices

Despite the differences in testing conditions and strategies of MP devices, numerous studies have compared their functions by performing testing using different microperimeters on the same subjects. In both SAP and MP, the task required of the subject is to distinguish the stimulus from its surrounding background. Where devices employ the same background luminance, their decibel scales can theoretically be aligned to each other by considering each unit of the respective scale in terms of their differential luminance value (calculated as background luminance subtracted from luminance at site of stimulus projection, described in detail by Parodi as well as Vujosevic) [63, 64]. For example, a differential luminance of approximately 127cd/m^2 corresponds to 4dB and 0dB on the MAIA and MP-1 mesopic decibel scales respectively. Thus, by adding 4dB to the MP-1 sensitivity value, one should arrive at the corresponding value in MAIA. In practice, this 4dB difference is often not observed, with substantial deviations evident[63, 65]. For example, patients with IRD with an average mean sensitivity (MS) of 5.68dB on MP-1 were found to have an average MS of 14.66dB in MAIA (instead of the expected 9.66dB)[63]. In the same study, normally sighted subjects with an average mesopic MS of 18.46dB on MP-1, had an observed average MAIA mesopic MS of 28.52dB (rather than 22.44dB). In pointwise sensitivity, an average difference of +7.3dB was found when comparing mesopic MP-1 to MAIA values in a mixed population group (normally sighted subjects and those with visual impairment)[65]. There was a 95% limit of agreement of -3.9 to 18.5dB, considered too wide-ranging to be of much clinical use. Similarly, in patients with AMD, a pointwise difference ranging between -14 and 6dB was seen in mesopic MAIA and MP-1 testing (although the median correction was MAIA = MP-1 +2dB)[66].

For device comparisons where the background luminances are not the same, Weber contrast (the differential luminance divided by the background luminance for that device) can be calculated for each unit of the respective device's scale. The decibel scales of the two devices is thus matched by the common scale of contrast values (i.e. the contrast value relating to a specified decibel value for one device is aligned to the same contrast value in the other device's decibel scale as explained e.g. by Liu et al[38]). Using this method, no difference in average thresholds, expressed as contrast values, was found between MAIA and Optos OCT/SLO in normal subjects [67]. This was not the case for the visually impaired patients in the same study and it was postulated that the brighter stimuli required may increase variability due to increased stray light effects. The OCT/SLO has also been compared with MP-1 via contrast values but found to correlate poorly [38]. It would be important to note that although one can theoretically align devices' scales according to contrast, different, not directly comparable physiological systems may be at work (e.g. mesopic with MP-1 and photopic with OCT/SLO).

However, MAIA and MP-3 utilize similar testing conditions but still generate differing mesopic retinal sensitivity values for normal subjects. Adding a 'correction factor' of 5.65dB to the MP-3 value to obtain the MAIA value allowed a strong statistically significant correlation to be demonstrated [68]. Possible explanations given for this disparity included differences in the systems used for stimulus projection and for grid placement onto the fundal image.

Reliability Indices

It is imperative that any clinical trial measurements are reliable and like SAP, MP offers indices against which the reliability of a test result can be gauged. However, in MP these indices are less evolved and not consistently available or applied.

In SAP, reliability indices refer to false positives, false negatives and fixation losses, whose assessment classically requires presentation of additional tests, so-called 'catch trials', typically making up 3-5% of stimuli presentations.[69] False positives refer to instances where a response is recorded when no stimulus is presented. These are either responses made when a stimulus is not presented but is anticipated to be, according to the expected ‘rhythm’ of stimuli presentation. Alternatively, the response time following stimuli presentation can be analyzed. The minimum response time to react to a stimulus is known to be around 180ms.[70] Adjusted for a subject’s mean response time, this period defines ‘response windows’ (when a response is expected to occur) and a ‘listening windows’ (when a response is not expected). Responses occurring in the ‘listening window’ are considered to be a false positive[71]. False negative catch trials involve presentation of suprathreshold stimuli at locations in which the threshold has already been determined. Fixation losses are characterised according to the Heijl-Krakau method which involves assessing the subject’s responses to stimuli presented at the optic nerve head.[72]

In MP, the situation is more fragmented. For instance, the MP-1S measures false positives by presenting stimuli at the optic nerve head whereas the MP-3 characterizes a false positive as a response made in the absence of a stimulus. The S-MAIA does not assess false positives or false negatives, but does provide an index referred to as ‘fixation losses’. However, these fixation losses are also assessed using optic nerve head stimuli presentations (with a 10dB intensity stimuli presented every 60 seconds when testing under full threshold, 4-2 strategy conditions). In fact, in the literature, researchers using MAIA often refer to this fixation loss metric as a false positive rate.[58, 60, 73] Generally, false negatives are not provided in MP devices but are available on the MP-3. A specific consideration for scotopic testing is that repeat testing and the presentation of suprathreshold stimuli may have the potential to disturb scotopic conditions.

It could also be argued that ‘fixation losses’ are not as relevant for MP as for SAP given that MP detects and compensates for retinal movements directly. This likely explains why researchers have moved away from this term, preferring the term false positive instead. Furthermore, accurate marking of the optic nerve head center is essential if fixation losses/false positives are to be accurately represented. This is because any off-center misplacement, especially in subjects with small optic discs, may render the stimulus visible due to stray light.

The S-MAIA manual states that fixation losses over 30% are unreliable. Published reports differ according to the level of fixation losses deemed tolerable, with research groups defining their own cut-offs such as 15%, 25% and 33%[74-76]. Given the small number of catch trials presented, one or two accidental button presses may be enough to classify an examination as unreliable. Available in later S-MAIA software versions, some groups have analyzed ‘wrong pressure event’ raw data as a surrogate for false positive rate, calculated as the number of wrong pressure events divided by the test duration.[58, 60] A wrong pressure event is a response occurring 1500ms or more after a stimulus presentation and prior to the next stimulus presentation.

The relative contributions of numerous S-MAIA reliability indices to variance in between-subject pointwise sensitivity (PWS) test-retest variability (TRTV) have been statistically explored in both neovascular AMD and GA.[58, 60] Parameters analyzed included false positive (blind spot presentation); wrong pressure event rate; examination duration time and fixation stability (95% bivariate contour ellipse area). In neovascular AMD, false positives were the most important factor for mesopic and scotopic red testing, whilst wrong pressure event rate had the greatest impact for scotopic cyan testing.[58] In those with GA, mean retinal sensitivity was the largest determinant of the variance of mesopic and scotopic cyan/red testing and wrong pressure event rate was more informative than false positives. This suggests indices other than false positive rate (termed 'fixation loss' by device) should also be considered when establishing inclusion criteria for test reliability in trial protocols. Such criteria may differ according to type of testing (mesopic, scotopic cyan/red) and pathology.

Microperimetry Retinal Sensitivity Indices & Analysis

The native software of microperimeters provide a limited range of retinal sensitivity indices. The most widely reported of these is mean sensitivity (MS): the arithmetic average sensitivity across all grid locations. Display of results also presents individual sensitivity values for each grid location (PWS) both numerically and visually, according to a color gradient. Given this limited range, research groups have maximised the use of raw retinal sensitivity data, devising alternative metrics of interest which feature heavily in interventional retinal disease trials as will become apparent shortly. These broadly fall into two categories: subdivisions of MS and PWS and scotoma evaluation. For quick reference, Table 2 summarizes both device and researcher-derived metrics. In addition, and as presented in Table 3, condition or treatment-specific characteristics and outcomes that have also been conceived by researchers and these will be discussed in relation to their pathology.

Mean and pointwise sensitivity

Taken at face value, MS is arguably a simple measure, however further reflection is warranted. As a global outcome, MS runs the risk of missing localized pathological variation in sensitivity[74, 77] as the difference in sensitivity across grid points is reduced by virtue of averaging. To retain some topographical information, MS may be calculated for subsections of a grid. An example of this is the categorization of a grid into central and paracentral areas, with MS calculated for each separately (CMS and PMS), as per Chen et. al.[78] Derivations of CMS and

PMS, varying by underlying grid design and expanse of central and paracentral areas, are commonly encountered in the literature.[79-87]

The constituent pointwise sensitivities should be examined to identify floor or ceiling effects, as in their presence they can cause the resultant MS to be over- or underestimated, potentially masking true change in MS across the tested region over time. Attempts have been made to account for floor effects by tracking the MS of only those points with a measurable threshold (i.e. non-absolute scotoma points).[88-90] Conversely, the approach of stratifying participants by baseline MS value has been used to mitigate ceiling effects, whereby changes in MS are separately examined in those whose MS is reduced at baseline.[81] To account for considerable variability in observed pointwise measures (i.e. scotomatous and non-scotomatous regions), Hood and colleagues have proposed calculating MS on a linear scale.[91] Conceptualised using SAP data in glaucoma, this method involves averaging anti-logged individual pointwise values before taking the log again.

MS is also inextricably influenced by the grid design. Total number of points, their spacing and their configuration will impact the information obtained. Commonly grids have more central than peripheral points, weighting MS in favor of foveal sensitivity. The use of different grids across studies also hinders direct comparison of MS. One method of addressing this is Hill-of-vision volumetric analysis such as that performed by Visual Field Modelling and Analysis (VFMA) software. Within the boundaries of a test grid, the operator can select a circular retinal area. The threshold sensitivities within this area are modelled to generate an interpolated volume sensitivity index, expressed in units of decibel-steradians (dB-sr) with higher values equating to better sensitivity. Although originally used with perimetry data, its use with MP raw data has been described more recently, including for Stargardt disease [27], achromatopsia [92, 93] and to evaluate the area of transplanted retinal pigment epithelium graft[94].

As with any measure, MS and PMS are subject to measurement error and variability. To be better equipped to distinguish disease progression from such variability, TRTV should be determined, ideally specific to the disease and device.

TRTV is conventionally defined by the 95% Bland-Altman Co-efficient of Repeatability (CoR) and is interpreted as a value for which 95% of test-retest differences for a subject are expected to fall, with smaller values indicating lower variability.[95] TRTV can be calculated for both PWS and MS. Understandably PWS CoR is higher than that of MS, as it does not profit from the averaging effects of the latter. As such there is a tradeoff between the precision of pointwise measures and repeatability. Table 4, although not exhaustive, is provided to familiarize the reader with the range of CoR that have been reported for various retinal conditions using different MP devices.

Scotoma

Using raw MP data, simplistic scotoma-driven outcomes have also been specified by research groups, such as the percentage or number of reduced, relative or absolute scotomatous points.[96-98] Cut-offs for what is considered reduced, relative and absolute loss vary across studies. Due to the customizable nature of MP testing grids, including differing stimuli counts and spacings used, care is advised in the interpretation of such metrics across trials. Repeatability of such measures has been described using a 37 stimuli grid in macula telangiectasia[99]. A CoR of 5 was found for absolute scotomatous points and 13 for normal sensitivity points (>25dB on MAIA). With variability representing 35% of the total scale in this case, the evaluation of the number of normal sensitivity points may be of limited use.

Fixation Stability

Microperimeters assess fixation stability throughout MP examinations or as standalone assessment. Fixation attempts are mapped onto the fundus image as a 'cloud' of points indicating the position and stability of fixation. The location of the 'cloud' reveals the retinal area used for fixation, the preferred retinal locus (PRL). The stability of fixation relates to the size of this area. Fuji *et al* described a method of quantifying fixation stability based on the percentage of fixation points within 2° and 4° diameter circles centred on the gravitational centre of all fixation points.[100] Fixation is categorised as 'stable' if more than 75% of fixation points fall within a 2° circle, 'relatively unstable' if fewer than 75% fall within a 2° circle, but more than 75% fall within a 4° circle and 'unstable' if fewer than 75% fall within a 4° circle.

First described by Steinman in 1965,[101] and reintroduced by Crossland *et al* in 2009[102], bivariate contour ellipse area (BCEA) has become a more prominently used measure to characterise fixation. BCEA is the area in minutes of arc^2 encompassing a defined percentage of fixation points, where higher values denote worse fixation. BCEA correlates more closely with reading speed and BCVA than the Fujii classifications. [102, 103] Though outside the scope of this review, fixation location and stability are also being investigated as potential endpoint measures, most notably in Stargardt disease.[104-106]

Review of microperimetry retinal sensitivity endpoints in interventional trials

In order to evaluate the adoption of MP retinal sensitivity as an endpoint in clinical trials to date, we conducted a literature review in Embase and Ovid Medline during September 2020. The results of two main searches were combined. First, MP free text search terms were combined with Boolean operator OR (microperimet*, fundus controlled perimet*, fundus-controlled perimet*, fundus automated perimet*, fundus-automated perimet*, retinal sensitivit*, macular sensitivity*). Perimetry was also included as a MeSH term. Second endpoint free text search terms were used combined with OR (endpoint*, outcome measure*). Clinical trial MeSH term was also included. The two search results were combined with AND. Only articles in English were considered and conference articles, or those using fixation stability only endpoints were excluded.

Studies were further categorized according to whether subjects had acquired or inherited retinal disease (IRD). Given the immense phenotypic variety in retinal disease and the impact this has on qualities such a repeatability and trial design, we considered inherited and acquired disease separately. Abstracts referring to acquired disease were reviewed to identify those describing randomized interventional studies stating MP retinal sensitivity as a primary or secondary endpoint. As randomized trials are not commonplace in IRD, all interventional IRD studies employing MP were retained. Tables 5 and 6 (Supplementary information) provide a summary of all articles reviewed in the IRD and acquired categories respectively, highlighting eye condition, study design, intervention, MP test parameters employed and endpoints utilised.

Inherited Retinal Disease

This resulted in the identification of 22 publications relating to interventional studies for IRD. Of these, one was excluded on account of it being a description of a single patient. From the remainder, there were 6 publications on choroideremia, 3 on Leber congenital amaurosis and 1 interventional study each for X-linked retinoschisis; X-linked Retinitis Pigmentosa due to defect in *RPGR*; *CNGA3*-related achromatopsia; autosomal dominant drusen and Stargardt disease. Five studies involved interventions in patients with retinitis pigmentosa of various genotypes, whilst one study involved patients with macular dystrophy and another described intervention in a mixed patient group including IRD. The studies are summarised in Table 5 and a selection of these will be discussed in further detail.

Choroideremia

Choroideremia is the ongoing target of ocular gene therapy intervention and numerous Phase I/II trials have published their results[28-30, 107].

As standard for Phase I/II trials, changes in BCVA and the occurrence of SAEs are predominantly cited as primary endpoints. Mesopic MP using the MAIA features prominently as a secondary endpoint. Interestingly, its use in some trials has taken the characteristics of the underlying condition into account by using customized grids.[28, 107]

A typical feature of choroideremia is the presence of scalloped patches of choroidal atrophy in the peripheral retina. These atrophic patches gradually enlarge, coalesce and encroach in a distinctive centripetal fashion on a central island of functioning retina. These areas can be tracked using FAF imaging where they appear hypofluorescent due to complete loss of overlying RPE.[108] In one Phase I/II gene therapy study, intact areas of retina were identified pre-intervention by FAF and MP grids created to fit these areas[107]. Alternatively, or in combination with custom grids, standard grids of varying degrees of coverage have been used within the same trial, the choice dependent on the size of the residual functioning retina within the same trial [28, 29, 109]. In studies comprising small numbers of participants with differing disease severity (and thus varying areas of intact retina), this tailored approach has its advantages. However, this does have implications for direct comparisons between eyes, between patients and across studies, and as the number of tested points vary, in averaging to obtain MS.

No statistically significant changes in retinal sensitivity measures in treated eyes versus untreated eyes or to baseline were demonstrated in these studies, although trends towards improvement in treated eyes were suggested from increases in BCVA and retinal sensitivity in some.[107] A range of additional parameters were also explored included 'peak' retinal sensitivity and total number of test points seen,[107] thus demonstrating the interest in defining additional metrics derived from analysis of the raw data to better determine intervention effects.

Leber Congenital Amaurosis

Numerous independent groups have reported the outcomes of AAV-mediated subretinal gene therapy intervention for Leber congenital amaurosis (LCA) caused by defects in the *RPE65* gene.[110-119] One has reported MP findings as a secondary endpoint, utilizing both central (68 points) and peripheral (55 points) MP-1 grids on each patient.[110, 120] During surgery, the retinotomy was made along the superotemporal arcade with the resultant bleb achieving foveal involvement in 10 of the 12 patients. The peripheral grid was positioned between 4 to 20° above fixation to cover the site of the retinotomy and its surrounding area. Changes in retinal sensitivity were reported according to the number of points which showed statistically significant improvement. Initial improvement in retinal sensitivity, assessed by MP was demonstrated in 5 treated eyes but appeared to decline from 6-12 months. A similar trend in dark-adapted perimetry-derived sensitivity measures was seen in 6 treated eyes. Such findings led to a new vector being developed to enhance potency and the potential for longer-lasting effects.[121]

The only other group (with the exception of an one patient account[122]) to report use of MP also used the MP-1 device, opting to report MS values and number of microscotomas, defined as points which were 0dB.[118] Both of these metrics were reported to be stable in both treated and untreated eyes. Other groups have emphasised other efficacy measures such as perimetry (kinetic and static) and perimetry-defined Hill-of-vision modelling metrics[119] or full-field sensitivity to assess retinal sensitivity over MP.[112, 113]

Discussion on *RPE65* LCA is not complete without reference to the first gene therapy product (Luxturna®; voretigene neparvovec-rzyl) to gain FDA and European Medicines Agency approvals and is also available as National Health Service (NHS) treatment in the U.K. In the initial stages, the primary endpoint was safety and a wide range of tests were used to evaluate visual function as the secondary endpoint.[114, 115] This included pupillary reflexes, nystagmus testing, perimetry, OCT changes, autofluorescence changes, full-field stimulus testing (FST), electroretinography (ERG), mobility testing and functional magnetic resonance imaging (fMRI). Notably, by Phase III, the primary endpoint was the change in vision-guided mobility performance under differing light levels at one year.[117] Therefore in these studies, tests other than MP were critical, particularly vision-guided mobility, given that nyctalopia is a pertinent feature of the disease.

X-linked Retinoschisis

X-linked retinoschisis is also the target of gene therapy, with intravitreal delivery favoured given the fragile condition of the retina and our literature search identified one study fulfilling our criteria. Cukras et al reported 18 month results of a Phase I/II trial of intravitreal AAV8-RS1 in nine patients.[31] Safety and the occurrence of inflammation were the primary endpoints. MP was used as one of the ways to evaluate retinal function as a secondary endpoint, with the authors analysing the raw data from MP-1 mesopic tests to categorise individual grid points as 'dense scotomatous' if the threshold sensitivity value was <0dB or 'responding' if otherwise. Grid points were further categorised according to whether they were 'extra-scotomatous' if separated from a dense scotomatous point by at least one other point, or 'para-scotomatous' if immediately adjacent. Given the limited dynamic range of the MP-1 device and the occurrence of floor effects, this type of sub-categorisation allowed data to be meaningfully assessed. MS of responding points, extra-scotomatous and para-scotomatous points were separately reported but no significant changes were demonstrated.

X-linked Retinitis Pigmentosa due to *RPGR* defect

There are multiple ongoing clinical trials assessing the effect of ocular gene therapy for retinitis pigmentosa secondary to defects in the *RPGR* gene (subretinal delivery in NCT03252847, NCT03316560, NCT03116113; intravitreal delivery in NCT04517149). One trial (NCT03116113) has published preliminary results of up to 6 months follow up, for which MP has featured prominently and the current Phase II/III of this trial has listed MP as a secondary endpoint[32]. Preliminary results of 18 patients included improvements in MS demonstrated in 6 patients under mesopic conditions using a standard 10-2 grid with the MAIA. Such gains were demonstrated in the medium and high vector dose cohorts, although the latter had a higher incidence (6 out of 9 patients in this cohort) of intraocular inflammation. MP results were presented using the standard device-generated interpolated color 'heat' maps of the sensitivity threshold at each tested point as well as a comparison of the number of points in which the stimulus was seen between treated/untreated eyes at baseline and at 6 months follow up. The course of inflammation in one high dose cohort patient was described in relation to the drop in MP retinal sensitivity value, corroborated by the patient's subjective symptoms of a paracentral scotoma and the development of subretinal hyperreflective lesions on OCT of presumed inflammatory origin. This was the clinical picture in the absence of a change in BCVA. Thus, MP helped to demonstrate efficacy as well as contribute to the clinical assessment of inflammatory complications in conjunction with other clinical findings.

Preliminary results from the *RPGR* gene therapy trials have also been made available as press releases, notably one of which (NCT03316560) refers to obtaining additional clarification from the FDA regarding clinically meaningful improvements using MP.[123] Initially the study group referred to responders as those who had shown an improvement beyond TRTV within the treated retinal area over at least two different visits (mesopic MP using MAIA).[124] More recently, they have gone on to define responders as those demonstrating improvement of at least 7dB in at least 5 points within the central area (centermost 36 points) of a 10-2 grid (consisting of a total of 68 points).[125]

***CNGA3*-Achromatopsia**

Achromatopsia due to defects in *CNGA3* is another area of active intense research in the field of ocular gene therapy, with multiple Phase I/II trials taking place concurrently across the world (NCT03758404; NCT02935517; NCT02610582). All trials list safety and the incidence of treatment-related adverse events as their primary endpoints. Trial NCT03758404 lists broad secondary endpoints of changes to BCVA, perimetry, MP and quality of life (QoL) measures. Secondary endpoints listed for trial NCT02935517 include changes to light aversion and color vision. Although the secondary endpoints listed for NCT02610582 refer to changes in visual function, their recently published 12month follow up results provide further details of the wide range of tests that have been used to characterise and monitor the patients' progress: BCVA; tests of spatial and temporal resolution; color discrimination; flicker fusion frequency; FST to red stimuli; contrast sensitivity (CS); pupillary responses,; QoL questionnaires and MP.[126] The group report the absence of any substantial safety concerns and noted that all nine adult patients who received subretinal injection of AAV8.CNGA3 had demonstrated some improvement in at least one secondary endpoint test. There was a statistically significant mean increase in BCVA of 2.9 letters and a CS gain of 0.33 log units in the treated eyes. However, MP changes (MS and fixation stability over 2^0 and 4^0) were not significant. Aside from these MP findings, the trial investigators have additionally utilized MP to track the PRL over time to confirm that at 1 year follow up, the PRL remains within the bleb boundaries of the treated macular area. Hill-of-vision analysis using the VFMA software had also been described in preliminary results with a modest improvement of 0.0613 dB-sr in the central 10^0 of the macula, but this was not statistically significant[93].

The use of such a broad range of tests to monitor the effect of the intervention is not an uncommon approach in such exploratory trials, given their early phase and the fact that many tests used to define clinical endpoints are yet to be fully established, including MP. In this trial, it is worth noting that the investigators also describe in detail how they set out to statistically combine 11 of the secondary endpoint tests to produce a single overall Z score, individualised for each patient.

Stargardt Disease

Although not an interventional trial, the use of MP in the study of Stargardt disease has been significant and will be briefly mentioned here. Stargardt disease is the most common cause of inherited macular dystrophy, affecting around 1 in 8000 to 10,0000 people, with autosomal recessive mutations in *ABCA4* accounting for the most common subtype, Stargardt type I (STGD1).[27] BCVA decline is slow, particularly in patients with older age at onset and thus other clinical endpoints to track early changes over time are being researched[127].

Structural metrics for disease progression include foveal outer retinal loss seen on OCT and changes to areas identified on FAF (typically a central area of hypofluorescence associated with RPE loss, surrounded by hyperfluorescence relating to lipofuscin accumulation).[8, 128] In terms of functional evaluation, MP is of particular interest given the typical eccentric fixation seen in this condition.

The Natural History of the Progression of Atrophy Secondary to Stargardt Disease (ProgStar) studies represent multicenter efforts to characterize and establish clinical endpoints for the condition. ProgStar consist of both retrospective and prospective longitudinal observational studies. To date they have reported findings from mesopic MP testing using the MP-1 in 359 eyes of 200 patients with STGD1 with one year of follow up. A 10-2 grid of 68 points was used (with customized spacing interval between points marginally distinct to that of the 2^0 spacing of the Humphrey 10-2 grid).

ProgStar defines a deep scotomatous point as one in which retinal sensitivity was 0dB or <0dB. It is worth noting other studies such as the previously described Cukras et al [31] define absolute scotoma as <0dB, as 0dB still signifies a response; a distinction important for interpretation of results. A relative scotoma was defined as a PWS value greater than 0dB but less than 12dB. Within these parameters, disease progression over one year was quantified in terms of decline in MS (-0.68dB); increase in number of deep scotoma points (+1.56 points) and a decrease in the number of points with a minimum retinal sensitivity of threshold 12dB (-3.01 points). MS were also provided for different grid subsections. Furthermore, custom software was used to automatically identify points adjacent to scotomas at the baseline visit to track over time.[129] This analysis revealed that the MS of points adjacent to scotomas undergo a faster rate of progression, highlighting their potential desirability as a clinical endpoint. It is thought that this may reflect higher disease activity occurring at the edges of the central atrophic area as it expands centrifugally. A previous ProgStar report had already established that longer disease duration was associated with worse MS and a greater number of deep scotoma points.[130]

Fixation metrics have also been studied in detail in the Progstar studies and changes in location of PRL and fixation areas quantified over one year[104]. However, hetereogeneity of changes were seen, perhaps reflecting the influence of neuronal adaptation that work to improve fixation; the existence of multiple PRLs and the need for longer follow up. Therefore, it was proposed that compared to retinal sensitivity, fixation metrics may be more suited as a secondary endpoint, with analysis focussed on a subset of patients.

The SMART (Scotopic Microperimetric Assessment of Rod function in Stargardt disease) is an ancillary study to Progstar that focuses on evaluating scotopic function using the MP-1S. Data were collected from 118 eyes of 118 participants though a different grid (composed of 40 points) was used for testing, thus limiting direct pointwise comparison with mesopic testing in these patients. MS in the first visit for mesopic and scotopic testing was 11.48 dB and 11.25dB respectively. However, the annual rate of decline calculated from longitudinal data analyzed over two years indicated that scotopic function deteriorated more than twice as quickly as mesopic function, with a loss of 1.42 dB, compared to 0.63dB per year respectively[131, 132] and as such may be a more sensitive endpoint for future trials. Moreover, an earlier study of scotopic function in 12 STGD1 patients demonstrated an association between scotopic sensitivity loss and structural changes on SD-OCT and confocal OCT.[133] In some cases, areas with normal structure were also observed to have reduced scotopic sensitivity.

In addition to studies of STGD1, the natural history of PROM-1 associated disease is currently being studied in Progstar-4 adopting both mesopic and scotopic microperimetry assessments.[134]

Acquired retinal disease

Our review resulted in 32 randomized interventional studies in acquired retinal disease, précised in Table 6, broadly classified as 11 in acquired macular disease [9 AMD, 1 myopic CNV and 1 macular telangiectasia]; 7 in diabetic eye disease [6 diabetic macular edema (DME) and 1 non-proliferative diabetic retinopathy (NPDR)]; 7 in vitreoretinal disease; 3 in CSCR; 3 in non-diabetic related macular edema (2 branch retinal vein occlusion and 1 uveitic) and 1 choroidal hemangioma.

Macular degeneration

MP has been used extensively in the study of macular disease in the last decades.[135-137] Though researchers in macular disease were amongst the first to recognize the benefits of MP, initially due to its compensation for poor fixation in GA,[138] more recently its potential as an endpoint has moved center stage.[6, 12, 13] Interest in mesopic and scotopic MP modalities has increased as their ability to capture subtle functional deficits in early and intermediate AMD[51, 59, 139-142] and quantify progression over time [143, 144] has been demonstrated. Of note, significant reductions in mesopic sensitivity over a period as short as 12 months have been shown,[73, 145] implying MP endpoints may allow for shorter trial durations.

This review identified 11 randomized interventional studies in acquired macular disease adopting MP-derived metrics as primary,[96, 97] secondary,[79, 98, 146-149] or exploratory endpoints.[150, 151] In 1 case the endpoint type was not clear.[152] Of particular note, 2 multicenter international randomized controlled trials successfully employed MAIA MP. The Macular Telangiectasia Type 2-Phase 2 CNTF Research Group recruited across 11 sites, collecting secondary endpoint data; whilst the LEAD Study Group recruited in 6 sites collecting exploratory endpoint data.

Interventions and conditions assessed by MP endpoints include lutein supplementation[79, 146] and oral telomerase for early AMD;[96] subthreshold micropulse laser in intermediate AMD;[150] photodynamic therapy (PDT) treatment regimens for age-related[97, 147] and myopic CNV;[151] intravitreal treatments for neovascular AMD;[148] suprachoroidal cell autograft[152] and lampalizumab[98] for GA and a ciliary neurotrophic factor (CNTF) implant in macular telangiectasia.[149]

To date, only mesopic MP has been included as a trial endpoint. Whilst earlier trials employed the MP-1, more recent studies have opted for the MAIA device. MS was the most commonly reported outcome. A range of other raw data defined metrics have been used; either modifications on MS or a surrogate for scotoma size.

Employed as a secondary endpoint, Huang et. al. described MS values over the central 1°, 3° and 5° in a randomized controlled trial (RCT) investigating potential functional benefits of lutein supplements.[79] Those taking either 10mg or 20mg of lutein had a greater increase in foveal sensitivity over 1° compared to a placebo group, an effect that was not evident when overall MS was considered.

In a multicenter RCT examining the effect of CNTF on retinal neurodegeneration in macular telangiectasia, aggregate sensitivity loss was reported as a post-hoc analysis[149]. Aggregate sensitivity loss considers both structure and function and relies on superimposition of SD-OCT and MP data. The technique was first described by Sallo using MP-1 data[153] and subsequently MAIA data.[154] Briefly, the inner segment/outer segment (IS/OS) break is defined on SD-OCT. Considering only stimuli within the central 10° of the grid, sensitivity values outside the IS/OS break are averaged and termed the background sensitivity. Individual sensitivity values of points falling within the IS/OS break are subtracted from the background sensitivity value. These differences are then summed and deducted from the background sensitivity value to give the aggregate sensitivity loss in dB. As such, aggregate sensitivity is a volumetric measure of scotoma depth and an example of a condition-specific, researcher-driven metric.

Other scotoma-based outcomes have also been used in relation to macular disease, such as the percentage or number of reduced, relative or absolute scotomatous points.[96-98] In general these metrics displayed concordance with the MS measures also reported in the individual studies. Further developing this theme, a longitudinal observational study of early and intermediate AMD plus normal controls compared the ability of several visual function outcomes to track progression over a 12 month period[145]. In addition to MS, percentage reduced threshold (PRT), expressed as the percentage of points falling below 25dB on MAIA testing was deduced. PRT was purported to be the most sensitive measure to map progression as it declined significantly in all 3 groups, over 6 and 12 months. The utility of MS of perilesional points and PRT as interventional trial endpoints have yet to be tested.

Though strictly falling outside the parameters of our search, novel potential treatments for GA have been assessed in Phase I/II and II studies, including neuroprotective agents, visual cycle inhibitors, immune modulating agents and antioxidants.[155] Frequently these trials have not included MP endpoints, presumably due to their early stage. However, the MP-1 was included as a secondary outcome in open label trials examining the safety and efficacy of the topically administered antioxidant OT-551,[88] and the immunosuppressive agent Sirolimus delivered subconjunctivally[89] and intravitreally.[90] Rather than report MS of all points examined, the average of all non-scotomatous points (defined at baseline) was calculated, thus minimizing floor effects of non-seeing retina. Ultimately efficacy of these treatments was not established, but these studies indicate MP has a place in future interventional trials in GA.

Additionally, utilizing MP data of those receiving OT-551 topically, additional GA-specific metrics were outlined.[156] Intended to track the progression of atrophy, Meleth evaluated both the number of scotomatous points (no response to brightest stimuli) and the MS of perilesional points (points immediately adjoining a scotomatous point). Significant per year progression was evident in each measure (+4.4 points and-1.20dB respectively, $p < 0.004$) suggesting promise as endpoints in future trials. In a similar vein, a novel deep scotoma mapping strategy using the physiological blind spot has been conceptualized in normal eyes.[157] Using 2 grids, the second with more points tightly spaced and centered on the optic nerve head, Wu and colleagues simulated scotoma progression. Their deep scotoma mapping strategy of probing the optic nerve head with single 10dB stimuli resulted in an almost 2-fold increase in the ability to detect simulated progression versus a standard 4-2 staircase approach. It was anecdotally more agreeable to subjects. Though additional validation is needed, deep scotoma mapping could improve the accuracy of tracking progression in atrophic retinal changes.

With respect to future alternative endpoints, reporting change in PWS is also likely to be important. PWS offers a more robust way of identifying local alterations and in combination with multimodal imaging has enhanced our understanding of specific functional deficits present with precise structural changes in AMD.[55, 143, 144] In fact, PWS over reticular pseudodrusen has been shown to exhibit faster progression than that detected in unremarkable retinal regions in the same eye. This effect was observed under mesopic and scotopic conditions using the MP-1S.[144] Similar analysis in eyes with large drusen demonstrated the same effect under the mesopic condition only.[143] Scotopic data in both studies was censored to some extent with the exclusion of participants who required a change of ND filter throughout the 3 year follow up period. On the basis they required a filter change, these eyes may be the ones experiencing the most change. Future studies on the MP-3S or S-MAIA, devices that do not rely on manipulation of ND filters and also have large dynamic ranges, will further enhance our understanding of longitudinal change in scotopic function in AMD.

The granularity with which we can functionally interrogate retinal lesions MP exposes some of the frailties of structurally defining disease severity. Pfau and colleagues, using mesopic and two color dark adapted MP, have demonstrated discrete functional phenotypes in eyes with cuticular, reticular and soft drusen which would all be classified as having intermediate AMD[55] Similarly, Hsu and coworkers have demonstrated longitudinal functional decline in MP measures despite no change in disease severity classification.[145]

Diabetic Macular Edema

Many landmark DME treatment trials over the last decade have not included MP endpoints, preferring BCVA and structural outcomes.[158-163] Nevertheless, we identified 5 randomized studies of laser and/or intravitreal drug treatments for DME listing MP as a primary,[80, 81] secondary [164, 165] or exploratory [82]endpoint, each using the MP-1 device. Where MP has been included as an endpoint to date, there is general consistency in device, test strategies and metrics, allowing for potentially easier cross trial comparison.

Vujosevic defined MS and foveal MS (FMS) over central 4° as primary endpoints in a single center trial comparing ETDRS laser photocoagulation to subthreshold micropulse diode laser in DME (SMDL)[80]. Significant improvements in MS and 4° FMS were observed only in those treated with SMDL, and the change between groups for both metrics was significant. In a later study of yellow versus infrared SMDL by the same group [81] using the same endpoints, no change in MS and 4° FMS was shown. However, stratifying results by baseline MS showed those whose baseline MS fell between 15 – 18 dB had significant within group improvements in MS and 4° FMS in both yellow and infrared SMDL groups. The limited dynamic range of the MP-1 leaves MS susceptible to ceiling effects. Stratification by baseline value mitigates this, particularly when baseline values are high.[81]

LUCIDATE, a single center RCT also adopted MS and 4° FMS, but as an exploratory endpoint in a study of Ranibizumab (RM) verses ETDRS laser in DME.[82] A subgroup of the Da Vinci study cohort, a multicenter RCT comparing doses and dosing regimens of intravitreal aflibercept (IA) to ETDRS laser[166] were examined by MP-1 to assess treatment related changes in MS.[164] A customized grid aligned with OCT subfields was used. MS was calculated in the central 4°, inner 10° and inner to outer ring (2° to 8° radius).

The Diabetic Retinopathy Research Group Vienna recently published MP-1 results[165] from a single center prospective randomized study of Bevacizumab versus Triamcinolone for

DME.[167] Presented as a secondary outcome in a standalone report, MP variables were defined as MS, absolute scotoma size [% of absolute (<0 dB) scotoma points] and relative scotoma size [% of relative (≥1 dB and < 10 dB) scotoma points]. MS significantly improved in bevacizumab treated eyes, mirrored by significant reductions in absolute and relative scotoma size.

As efforts to find new therapies in DME continue, early phase trials have included MS metrics as secondary endpoints, notably in a Phase I/II trial of oral Dextromethorphan[168] and a Phase II trial of Cibinetide.[169] We are unaware of test-retest values derived from DME cohorts which may hamper understanding of the minimal change thought to be clinically significant. Undertaking this preparatory work could help define the value of MP as an endpoint in DME particularly given its inclusion in these recent early phase studies.

Given mesopic retinal sensitivity deficits have been identified in diabetes prior to the development of diabetic retinopathy,[170] MP may be a candidate endpoint as treatments are developed for earlier disease. In fact, MAIA-derived mesopic MS and Macular Integrity Index were defined as primary endpoints in a non-randomized prospective controlled study of Docosahexaenoic acid supplementation in non-proliferative diabetic retinopathy.[171] Though mesopic measures may hold promise, scotopic MP has not identified rod-based functional deficits in diabetic eyes with or without non-proliferative diabetic retinopathy[172] and so focus will likely remain on mesopic measures.

Central Serous Chorioretinopathy

Three randomized studies of CSCR treatments were identified, each reporting a structural primary endpoint and mesopic MS as a secondary endpoint [173-175]. No other MP metrics were reported.

The efficacy of half-dose PDT over High-Density Subthreshold Micropulse Laser (HSML) in chronic CSCR was established in a large multicenter RCT, the PLACE trial, undertaken at 5 academic medical centers across Europe.[173] Primary outcome was resolution of subretinal fluid (SRF), whilst secondary functional endpoints were functional (BCVA and MS).[176] SRF resolved in significantly more eyes receiving half-dose PDT than HSML. Concordant changes in visual function were demonstrated with half -dose PDT patients showing a significantly higher increase in BCVA and MS.

Data on a subgroup of PLACE subjects with persistent SRF at study conclusion were recently published in the very aptly named REPLACE crossover trial.[174] Crossover to half-dose PDT group showed significant improvement in MS, without improvement in BCVA.

Successful use of MP in a large international multicenter trial setting is significant. However MP testing strategies were not specified beyond acknowledging examinations were performed on 2 devices (MP-1 and MAIA), with subjects followed up on the same device. Measurement scales were aligned using a conversion method described by Parodi et al in a small cross sectional pilot study of eyes with IRD and normal control.[63]

Vitreoretinal surgery

Seven randomized studies of vitreomacular surgery outcomes and techniques were identified using mesopic MP measures as primary [86, 87] or secondary [83-85, 177, 178] endpoints. MP-1, MAIA and OCT/SLO devices were used. In addition to MS, measures of foveal function over the central 2° [86] and 4° [83-85, 87] and number of absolute scotoma points[87] have been defined as outcome measures in randomized vitreoretinal surgery studies.

Of particular note, a multicenter RCT comparing the merits of ILM peel during vitrectomy for idiopathic macular pucker using 4° FMS as a primary endpoint, revealed significantly better foveal function in eyes without ILM peel, despite no difference in BCVA between treatment groups.[87]

FMS over 4° has also been used as a secondary endpoint in single center randomized studies comparing outcomes of complete versus foveal sparing ILM peels in both macular hole surgery[83, 84] and epiretinal membrane removal.[85] Change in BCVA, the primary outcome in these three studies, was not significantly different between groups, whereas change in 4° FMS was significantly higher in the foveal sparing arms of all three studies. Though FMS has been shown to have a significant moderate correlation to BCVA in eyes undergoing vitreomacular surgery,[86] these results suggest FMS may be better able to describe changes in foveal function following vitreomacular surgery than BCVA alone.

Conclusion

While MP is yet to be fully established as a clinical trial endpoint, undoubtedly there is abundant interest in its utility as such, underlined by the scope of its uptake demonstrated in this review, including in endpoint development studies [8, 12, 13, 15] as well as the seemingly countless ways in which novel metrics from raw data are being conceived. Where MP has been taken up as an endpoint in both inherited and acquired retinal disease, it has predominantly been used as a secondary outcome to date. BCVA persists as the main functional outcome measure of choice, however we did see notable exceptions in AMD, [96, 97] macular edema,[80, 81, 179] and even vitreoretinal surgery.[86, 87]

In addition to interest in and uptake of MP, this review illustrates the extensive variation in how it is being employed in terms of device, test strategy and reported metrics. Given the breadth and heterogeneity of retinal conditions, it would be unrealistic to expect one optimal test strategy or all-encompassing metric. More credible is the concept of condition or treatment-specific approaches. The custom features of MP devices provide a fertile environment for this. As illustrated in Table 3, examples of MP features being used in this way are frequently seen, be that via grid customization in choroideremia or surgical procedures; or exploiting raw data to create condition-targeted indices as in GA and Stargardt disease.

Whilst tailoring an exam to a treatment area, lesion or expected drug effect is desirable, clinical trial endpoints are by their very nature required to be standardized. It is of course possible to standardize what was once custom, however without more overlap in strategies and reporting, it may be difficult to accumulate a sufficient body of evidence to validate a particular strategy. The current lack of consensus may be stifling the development of well-defined MP endpoints and the opportunity to compare results across trials. Transparent and detailed reporting of test strategies, especially where customization is relied upon and novel metrics used, is a must.

Endpoint development for MP is still in its infancy, however achievements in SAP glaucoma analysis may guide its next steps. There have already been explicit attempts to replicate SAP visual field indices in retinal conditions using MP data. Pattern deviation; total deviation; mean defect; mean deviation; pattern standard deviation and loss variance have been evaluated in recent studies[76, 180]. Cluster analysis has also been used to describe disease-specific patterns of visual field defects[180]. Although intuitive in glaucoma, this type of functional grouping may not be so readily achieved for retinal disease given its heterogeneity. It remains to be seen which indices may be adopted and for which retinal diseases. What would be of great practical benefit is the development of software that performs automated statistical analysis, like the Glaucoma Change Probability (GCP) software, which compares pointwise changes with an averaged baseline (often from 2 or 3 tests) and flags up changes that exceed the expected variability.[181] The robust establishment of normative data, together with TRTV data, as discussed later, are essential prerequisites for this.

To our best knowledge, regulatory authorities currently do not recognise any MP metrics as clinical trial endpoints. Yet again, the example of SAP may provide insight into what regulators may reasonably expect. For instance, the FDA and National Eye Institute Glaucoma Clinical Trial Design and Endpoints Symposium suggested visual field progression may be an adequate primary endpoint; specifically a between-group difference in visual field progression with 5 or more points showing significant changes from baseline or a statistically and clinically significant between-group difference across the total visual field, purported to be 7dB[182]. Therefore, we anticipate that any MP endpoint metrics will involve stipulations on number of points demonstrating change and an established threshold sensitivity value to cross, presumably according to disease and device used.

A discussion of endpoints would not be complete without consideration of the practical elements of implementing said endpoint in a multicenter clinical trial setting. No amount of repeatability or sensitivity can confirm the value of an endpoint if it is impractical to measure. MP has a reputation of being a lengthy, burdensome test for patients and operators alike. Even as devices have become more automated and test durations shorter, this perception has persisted,[183, 184] and a recent study cited patient refusal to complete MP as limiting longitudinal data collection.[145] Though high quality MP data can certainly be obtained in laboratory and small clinical study settings by motivated researchers and clinicians, it remains to be seen whether this can be scaled up appropriately, but there are positive signs.

The LEAD study, though designating MP as an exploratory endpoint, should be commended for successfully coordinating MAIA data collection for 280 subjects with intermediate AMD at 5 Australian and 1 Northern Irish site in an interventional RCT; a very significant achievement. Adding further weight to the viability of MP in large scale trials, natural history study ProgStar recently published 12 month follow up MP-1 data on 359 eyes with Stargardt disease from 9 sites across The United States and Europe[185], a very meaningful accomplishment given the significant visual impairment of this cohort.

That being said, pivotal trials for anti-vascular endothelial growth factor in neovascular AMD[3-5] and DME[158, 160] recruited at 70 to 150 sites across international borders. Even if, as hoped, more sensitive validated endpoints make smaller, faster trials a reality, it is still exceedingly likely that trials for novel treatments in high prevalence conditions such as AMD or DME will be conducted at a large number of sites. As yet, it has not been shown whether a large, high quality MP data set can be acquired under such circumstances. Each with 20 international sites, MACUSTAR[12] and the AMD Ryan Initiative Study (ARIS)[14] will offer further insight into this within the context of AMD. In contrast, gene therapy trials typically recruit at a small number of specialist tertiary centers, potentially making practical considerations somewhat easier to manage.

MP requires trained, skilled operators, with each site needing a least 2 personnel depending on the size of the trial. Operators should be certified as being able to perform the test to the required standard. This is usually assessed by adherence to a standard operating procedure (SOP), reliability indices and image quality of a set number of examinations on normal eyes and eyes with the pathology under investigation. Clinical experience has shown us that engagement with operators and clinical sites, especially if new to the technique, is essential. A proficient, confident operator stands the best chance of capturing accurate data and making the examination acceptable to patients. After all, a primary endpoint assessment that too few patients can complete is not viable.

Knowing how to technically operate the instrument though important, is not the only consideration. Patient instruction needs to be clear, concise and consistent. Though newer devices are more automated, operators need to keep patients engaged and focused throughout the examination, whilst remaining reactive to signs that compliance is waning, such as wandering fixation or closing eyelids. Positive, constructive and ongoing feedback is key. Regular data quality reviews should be implemented and feedback provided to operators and sites wherever protocol deviations or missing data are observed and of equal importance, when data quality is high.

Endorsement of scotopic MP as an endpoint brings added challenges. A period of at least 30 minutes of DA is a prerequisite for scotopic testing, requiring patients to sit in light-tight, dark room conditions. This needs a windowless room, with a light-tight seal around the door and any artificial light sources (e.g.computer screens, power light, exits signs) within the room need to be disabled or covered by a long wavelength red filter. It is important to emphasis the distinction between these conditions and for instance, a cubicle with dimmed light adequate for SAP. Many clinical trial centers will not have ready access to suitable dark room conditions. Prior planning and organization will likely be necessary, as well as some form of monitoring to ensure appropriate conditions are achieved and maintained for the duration of the study. From commencement of DA to completion of testing, the dark room conditions must be preserved. If light enters the room during testing, the data collected will not be valid. This, combined with the specificity of the conditions dictates that a dedicated, sole purpose room is desirable.

The experience of total darkness can be unpleasant. This impacts on patients and operators alike, who will both be required to remain within these conditions for the entire DA and testing period. Again, the skill and reassurance of the operator will be key in ensuring these circumstances can be tolerated by patients. Adherence to a full period of DA is mandatory for data validity. A means of ensuring adherence across all trial sites should be implemented.

Though not insurmountable, implementation of scotopic testing on a large scale certainly has test-specific challenges to manage.

Despite there being no examples of scotopic MP being employed as a primary or secondary endpoint in an interventional clinical trial as yet, it is very encouraging that the SMART study, which scrutinises the potential role of scotopic MP as an endpoint in Stargardt disease has recruited 118 participants[57]. Furthermore, scotopic MP has also been included in endpoint development studies in AMD.[12-14]

Adopting MP as a primary endpoint on a large multicenter clinical trial will also impact budget. Commercially available microperimeters are expensive and a clinical reading center will be necessary to provide standardized, objective, anonymized grading of results.[186] Arguably costs may be similar to those incurred when employing imaging modalities such as OCT. However, should MP replace existing functional assessments which require minimal equipment and no external grading such as BCVA, the cost differential is likely to be substantial.

Given the choice of commercially available devices, the decision of which instrument to use in a trial also requires considerable thought. It is certainly not desirable to use more than one device during a trial given incompatibility of dB scales across devices. Therefore, a high level of upfront commitment is necessary. Deliberation should include whether analysis will require comparison with a normative database; which stimuli and grid settings may be most appropriate; under which luminance conditions retinal sensitivity should be measured and how the patient population of interest will fare on a given device or test strategy. That said, researchers may deliberatively want to match test settings and pre-test DA protocols to allow direct comparison across interventions. In addition to new instruments becoming available, software and hardware updates are periodically released bringing in new features and phasing out others. The impact of such updates on trial data collection should therefore be established before implementing any changes.

Yet further still, during the course of a clinical study, the pros and cons of particular devices and test strategies may become apparent. If implementing MP in a patient population for the first time, piloting testing is advisable. Instrument costs likely prohibit piloting different devices, but the option of trialling differing test strategies is feasible. This also allows for determination of TRTV in the patient population under investigation, an approach previously used in AMD,[187] XLRS[188] and *RPGR* Retinitis Pigmentosa.[189] Intuitively it is expected that TRTV may vary with baseline retinal sensitivity. Though this effect has been observed,[99] so too has independence of baseline sensitivity from TRTV.[190]

The results of TRTV studies across a breadth of retinal disease introduce further considerations for trialists. CoR is often presented as a threshold change that is clinically meaningful as a smaller change may be considered measurement error.[99, 188, 189, 191] However caution has been urged in defining treatment response related to TRTV variability without taking interexaminer effects into account[78] as it is highly likely that multiple operators may perform MP assessments over the course of clinical trial. Furthermore, if TRTV is defined in eyes with a pre-existing dense scotoma, PWS CoR may be inflated due to the increased variability of PWS on the scotoma edge.[77] In this case, the use of PWS CoR as the threshold for clinically significant change may be setting the bar too high.

Whether TRTV is assessed with or without follow-up mode enabled will likely impact on CoR. The use of follow-up mode ensures the same retinal locations are examined on retesting, which in dedicated endpoint exploring studies is ideal. However, patients enrolled in interventional trials often perform repeat testing as part of their baseline assessment. If follow up mode was enabled in these cases, this would result in the selection of a pre-intervention test as follow-up. To avoid this, researchers may decide to perform pre-intervention baseline assessments without the use of follow-up mode but this is likely to result in higher TRTV values being obtained. Additionally, in follow-up mode, the starting stimulus intensity at any given point is informed by the values obtained in the baseline test (either at or near the baseline value), thus contributing to a shorter examination duration.[52]

The presence of learning effects in MP has also been explored extensively within TRTV studies, the results of which have implications for clinical trial design. Learning effects have been confirmed in those without prior experience of MP, culminating in improved performance on a repeat test,[74, 99] with authors advocating that the first examination be considered practice only. Conversely, in other studies learning effects have not been observed, although a truncated practice examination was performed prior to testing in these cases.[59, 191] Despite such disparities, it is recommended MP protocols include some form of practice session or exam before baseline testing.

Given some gene therapies may target IRDs in children, it is encouraging that the viability of MAIA testing in children with normal vision between the ages of 9 and 12 years has been reported. [192] However, in comparison to adults with normal vision, CoR was significantly higher and averaging of multiple tests was advised. Further work to establish TRTV limits in children with IRD is warranted.

In addition to considering retinal sensitivity in isolation, the utility of composite endpoints incorporating MP has also been raised. A composite endpoint generally comprises multiple single independent endpoints which on their own may not possess sufficient reliability or sensitivity, but do so in combination.[193] Using SAP, a combined structure and function index has been shown to perform better than isolated measures in glaucoma detection and staging.[194] Indeed the diagnostic ability of such an index performs better in eyes with field loss when MP versus SAP is used.[195] A similar structure-function approach has been suggested for future *ABCA4* trials[196] and composite approaches incorporating MP have been proposed in CSCR,[197] AMD[198] and IRD generally.[132]

Moreover, the potential of numerous OCT-defined structural indices to act as surrogate biomarkers for retinal sensitivity have also been reported, specifically in AMD, DME, macular telangiectasia and Stargardt disease. Across this spectrum, ellipsoid zone loss/integrity; retinal pigment epithelium drusen complex; hyper reflective loci; outer retinal thickness; reticular pseudodrusen; nascent GA and pigment clumping have all shown promise as retinal sensitivity biomarkers.[8, 144, 199-204] Of course, surrogate structural endpoints will only be of interest if shown to be associated with visual function loss.[1] Furthermore, artificial intelligence has brought exciting innovation to this field. Deep learning models have been developed that can reliably predict or 'infer' mesopic and scotopic retinal sensitivity based on imaging data alone in AMD[183] and macular telangiectasia.[184] Although further validation is necessary, these are exciting new avenues to explore.

If, as we all hope, novel interventions for retinal disease are established, recipients of such therapies will need to be monitored and assessed for treatment response in routine clinical practice. Indeed, one of the great successes of SAP has been its crossover to routine clinical use; it is almost universally available, frequently repeated in patients and familiar to clinicians. The same cannot be said of MP currently, and even with time and ensuing familiarity, such practical considerations, like the ones we have described, may impact its crossover from research to clinical practice. However, if structural biomarkers and / or AI derived pseudo functional outcomes were to be validated, hypothetically a single objective OCT scan could replace mesopic and scotopic MP examinations in the future. This has the potential to transform clinical trial design, reducing patient burden, equipment costs and, via frequent, early data capture, study durations.

In summary, despite the current lack of consensus, there are encouraging signs that MP may deliver on the promise of endpoint validity. Endpoint development trials will undoubtedly be key in understanding the validity of microperimetry as a clinical trial endpoint, but existing signs are promising.

Statements

Acknowledgement (optional)

We would like to gratefully acknowledge (in alphabetical order): Mauro Campigotto (NIDEK Technologies, Padova Italy), Marco Morales (former co-founder of CenterVue, Padova, Italy) and Carlo Pellizzari (CenterVue, Padova, Italy) for their technical advice and clarifications.

Conflict of Interest Statement

Yesa Yang: None

Hannah Dunbar: Boehringer-Ingelheim

Funding Sources

Yesa Yang: None

Hannah Dunbar: time funded from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 116076. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

Author Contributions

Yesa Yang: Conceptualization, Methodology, Analysis, Writing-original draft, Writing-review & editing

Hannah Dunbar: Conceptualization, Methodology, Analysis, Writing-original draft, Writing-review & editing

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	Nidek MP-1/ MP-1S*	**Nidek MP-3/ MP-3S***	**MAIA/S-MAIA* 2nd generation**	**Optos OCT/SLO**	**Compass**
Background luminance	Mesopic: 1.27cd/m^2 Scotopic: 0.0025cd/m^2	Photopic: 10cd/m^2 Mesopic: 1.27cd/m^2 Scotopic: 0.00095cd/m^2	Mesopic: 1.27 cd/m^2 Scotopic: <0.0001 cd/m^2	Photopic: 10 cd/m^2	Photopic: 10cd/m^2
Maximum stimulus intensity	Mesopic: 128 cd/m^2 Scotopic: 0.25 cd/m^2	Photopic: 3183.1cd/m^2 Mesopic: 319.58/m^2 Scotopic: 0.096cd/m^2	Mesopic: 318cd/m^2 Scotopic: 2.54 scotopic cd/m^{2**}	125cd/m^2	3183.1cd/m^2
Dynamic Range	0-20dB	0-34dB (photopic & mesopic) 0-24dB (scotopic)	0-36dB	0-20dB	0-50dB
Fundus Field of View	45^o	45^o	36^o	29.7^o	60^o
Fundus Image	B&W IR (live feedback) Colour (results display)	B&W IR (live feedback) Colour (results display)	B&W SLO	B&W SLO	Colour, IR, Red-free
Fundus Image resolution	768 x576 pixels (B&W); 1392 x 1038 pixels (Colour)	768 x576 pixels (B&W); 4290 x 2800 pixels (Colour)	1024x1024 pixels	512x512 pixels	2592x1944 pixels
Threshold Strategy	4-2; 4-2-1 Staircase, & others including manual	4-2; 4-2-1 Staircase	4-2 Staircase Suprathreshold tests	4-2; 4-2-1 Staircase & others including Suprathreshold	4-2, ZEST
Stimulus Duration	100-2000ms	100 ms, 200ms	200ms	200ms, 300ms	200ms
Stimulus Size	Goldmann I to V	Goldmann I to V	Goldmann III	Goldmann I to V	Goldman III
Normative data	Provided for mesopic (local defect maps)	Absent	Provided for standard grid use in mesopic (Macular integrity index)	Absent	Provided
Fixation tracking speed	25Hz	30Hz	25Hz	8Hz	25Hz

Biofeedback training	Yes	Yes	Yes	No	No
Importing of Images	Yes (images & OCT)	No	No	Yes (OCT)	No

Table 1: Summary of characteristics of commercially available Microperimeters
*: References to scotopic features in table relate to the scotopic version of the device; **: units based on scotopic luminosity function; B&W: Black & White; IR: Infrared; ms: milliseconds; ZEST: Zippy Estimation by Sequential Testing

Endpoint metrics provided by device	**Researcher-derived metrics from raw data**
Mean sensitivity - Mean of all points in a test grid Pointwise sensitivity - Individual sensitivity at each point Interpolated colour 'heat maps' - PWS values expressed according to a colour gradient, superimposed on fundus image	Mean sensitivity of subsections of grid - based in eccentricity from fovea i.e. CMS or PMS - of non-scotomatous points (defined at baseline) Change in pointwise sensitivity - change to pointwise sensitivity over time - change in number of points reaching a certain threshold sensitivity value* Scotoma size defined by number or % of - absolute scotoma points* - relative scotoma points* Number of 'seeing' versus 'non-seeing' points Volumetric indices derived using hill of vision modelling software

Table 2: Microperimetry retinal sensitivity metrics provided by device compared to researcher-derived metrics in the literature
*cut-offs for relative and absolute vary by study
CMS: central mean sensitivity; PMS: paracentral mean sensitivity

Condition	Customization of MP settings
Choroideremia	Grid customized according to intact retina identified by FAF
Targeted treated areas (e.g. gene therapy vector bleb; area of stem cell graft)	Grid customized to demarcate treated versus untreated areas for direct comparison
Stargardt Disease	MS of edge of scotoma points
Early / intermediate AMD	PWS over specific retinal lesion identified on OCT (i.e. reticular pseudodrusen, large drusen, nascent GA) versus unremarkable regions Percent reduced threshold (% of points with abnormal retinal sensitivity defined as < 25 dB on MAIA)
Geographic Atrophy	MS of peri-lesional points (points immediately adjoining a point where brightest stimuli unseen) Deep scotoma mapping strategy
Macular Telangiectasia	Aggregate sensitivity loss
Diabetic Macular Oedema	MS over OCT subfields

Table 3: Examples of MP features and raw data used to define condition or treatment specific metrics

AMD: Age-related macular degeneration; FAF: Fundus Autofluorescence; GA: geographic atrophy; OCT: Optical Coherence Tomography

Condition	Device	Test	PWS CoR	MS CoR
Mixed macular diseases Chen et al, 2009[78]	MP-1	Mesopic	5.56dB (4.95dB if floor/ceiling effects removed)	1.81dB
Stargardt Disease Cideciyan et al, 2012 [190]	MP-1 (custom grid; red stimulus)	Mesopic	4.2dB	N/A
XLRS Jeffrey et al. 2014 [188]	MP-1	Mesopic	6.8dB (better eye) 5.4dB (worse eye) Floor/Ceiling effects removed	2.2dB (better eye) 1.7dB (worse eye) Floor/Ceiling effects removed
Macular telangiectasia Wong, et al 2017[99]	MAIA	Mesopic	7.20dB	2.60dB
Intermediate AMD Welker et al, 2018[59]	S-MAIA	Mesopic Scotopic	4.40dB 4.52dB	N/A
RPGR Retinitis Pigmentosa Buckley et al. 2020 [189]	MAIA	Mesopic	6dB	1.30dB

Table 4: Examples of 95% co-efficients of repeatability for a variety of retinal disease reported in literature.
PWS: Pointwise sensitivity; CoR: 95% co-efficients of repeatability; MS: mean sensitivity; dB; decibel; AMD: age-related macular degeneration; XLRS: X-Linked Retinoschisis; N/A: Not applicable/available

Study	Study Design & Interventions	Condition	Microperimetry test parameters	Endpoints
MacLaren et al., 2014 [107] NCT01461213	Phase I/II Subretinal AAV2.REP1	Choroideremia	MAIA Mesopic 20 min DA Custom grid tailored to intact macular areas identified on FAF	<u>Primary</u> BCVA <u>Secondary</u> MP: -Change in maximal point sensitivity -Changes in MS -Dimmest stimulus seen -Total no. of points seen OCT thickness FAF area
Xue et al., 2018 [28] NCT01461213 (final outcome of [107])	Phase I/II Subretinal AAV2.REP1	Choroideremia	MAIA Mesopic 20 min DA Followed protocol in [107] but also 10º & 20º grids used in some pts, according to floor effects encountered	<u>Primary</u> BCVA <u>Secondary</u> MS OCT-retinal thickness FAF area
Dimopoulos et al., 2018 [29] NCT02077361	Phase I Subretinal AAV2.REP1	Choroideremia	MAIA Mesopic 20 min DA Standard grid of 37 points for 5 pts 10-2 grid of 61 points for 1 pt	<u>Primary</u> Safety (AEs, & assessed by OCT, FAF) <u>Secondary</u> BCVA MS Areas of intact RPE on FAF
Lam et al., 2019 [30] NCT02553135	Phase II Subretinal AAV2.REP1	Choroideremia	MAIA Mesopic Grid not specified	<u>Primary</u> BCVA, AEs <u>Secondary</u> MS FAF area OCT parameters
Fischer et al., 2019	Phase II	Choroideremia	MAIA	<u>Primary</u>

[205] NCT02671539 (24 month data of [109])	Randomization of eye Subretinal AAV2.REP1		Mesopic 30 min DA 10-2 grid with 68 points	BCVA <u>Secondary</u> MS FAF changes OCT parameters
Fischer et al., 2020 [109] NCT02671539 (12 month data with focus on retinal sensitivity)	Phase II Randomization of eye Subretinal AAV2.REP1	Choroideremia	MAIA Mesopic 10-2 grid with 68 points (if <6 points seen on above grid, a 37 point, 10º coverage grid was used)	<u>Primary</u> BCVA, safety <u>Secondary</u> MS Maximal point retinal sensitivity FAF changes OCT parameters
Bainbridge et al., 2008 [111] Bainbridge et al., 2015[110] (Final outcome of [111]) NCT00643747	Phase I/II Subretinal AAV2/2.hRPE65p.hRPE65	Leber congenital amaurosis	MP-1 Mesopic 10min DA 55 point grid, appears to be positioned over superotemporal arcade (site of retinotomy)[111] 2 types of grids used in all pts: Central (68 pts) & Peripheral [110] Goldmann V 4-2 staircase	<u>Primary</u> Inflammation, AEs <u>Secondary</u> Visual function [BCVA, kinetic perimetry, MP (pointwise sensitivity), DA perimetry, mobility, CS, color vision, spectral sensitivity, retinal imaging ERG]
Le Meur et al., 2018 [118] NCT01496040	Phase I/II Subretinal AAV2 or AAV4 RPE65-RPE65	Leber congenital amaurosis	MP-1 Mesopic 10 min DA Grid not specified	<u>Primary</u> AEs, biodistribution of viral vectors (in urine, nasal samples, blood)

				Secondary ERG, BCVA, pupillometry, MP – MS; No. of microscotomas Mobility
Cukras et al., 2018 [31] NCT02317887	Phase I/II Intravitreal AAV8-RS1	XLRS	MP-1 Mesopic 68 points 10-2 grid	Primary AEs, inflammation Secondary Visual function, ERG, AAV antibodies, OCT changes MP –no of points which did not reach floor vs floor effects (dense scotomatous points); MS of responding points MS of extra-scotomatous points MS of para-scotomatous points.
Cehajic-Kapetanovic et al., 2020 [32] NCT03116113	Phase I/II Subretinal AAV8-codon optimised *RPGR*	*RPGR* RP	MAIA Mesopic 68 points 10-2 grid	Primary Safety Secondary BCVA, MS, central retinal thickness
Fischer et al., 2020 [126] NCT02610582	Phase I/II Subretinal AAV8.CNGA3	*CNGA3* Achromatopsia	MP-1 Mesopic 20º grid	Primary Safety, inflammation Secondary Change in visual function (BCVA, MS, 2º & 4º fixation stability, spatial & temporal resolution, chromatic tests, flicker fusion frequency, CS, pupillary responses, FST, QoL)
Lenassi et al., 2013 [206]	Prospective, interventional case series Argon green laser to RPE anterior to drusen	Autosomal dominant drusen (EFEMP1-related maculopathy)	MP-1 Mesopic Humphrey 10 Program (76 points, central 20º) 4-2 staircase	Primary BCVA Changes in PWS Scotoma size (no. of points <0dB) Drusen volume on OCT Secondary

				Safety, development of CNV
Mehat et al., 2018 [94] NCT01469832	Phase I/II Subretinal transplantation of hESC-derived RPE	STGD1	MP-1 Mesopic Central 20º (including coverage over transplanted area) Also a high density grid to analyse transplanted area	Primary Safety, tolerance Secondary Retinal structure & function by MP (PWS, Hill of vision modelling using VFMA) OCT, perimetry (static & kinetic), mERG
Yamamoto et al., 2012 [207] UMIN-CTR Clinical Trials number: JapicCTI-090748	Phase II Randomised, double-blind, placebo-controlled Topical Isopropyl unoprostone Placebo	RP (clinical diagnosis)	MP-1 Mesopic No DA 24 points 10º grid 4-2 double staircase	Primary Change in central 2º retinal sensitivity (specifically no. of points with ≥4dB decrease) Secondary BCVA CS Change in 10º retinal sensitivity Mean deviation in HFA QoL
Tawada et al., 2013 [208]	Non-comparative pilot study Topical isopropyl unoprostone	RP (clinical diagnosis)	MP-1 Mesopic No DA 10º (24 points) 4-2 staircase 3° red single cross fixation target	Primary Change to central 2º retinal sensitivity Secondary BCVA MP (MS; central 10 º MS; No of points improved by ≥2dB and ≥ 4dB) Perimetry (MD on HFA 10-2)
Wagner et al., 2017 [209] NCT01847365	Single-arm open label interventional safety trial Weekly transcorneal electrical stimulation	RP (varying genotypes)	MAIA Mesopic 20 min DA	Primary Safety Secondary

			10-2 grid	Efficacy according to structure & function [BCVA, MP (MS) or Goldmann VF]
Campochiaro et al., 2020 [210] NCT03063021	Phase I Oral *N*-acetylcysteine	RP (clinical diagnosis, varying genotypes)	MAIA Mesopic 68 points 10-2 grid	Primary Safety, tolerance Secondary BCVA, MS, EZ width, aqueous NAC
Kong et al., 2020 [211] NCT03063021 (This is a point wise analysis of data from [210])	Phase I Dose escalation, single-centre, open-label Oral *N*-acetylcysteine	RP (clinical diagnosis, varying genotypes)	MAIA Mesopic 68 points Central 20º	Primary Safety Secondary Visual function: BCVA, MP – PWS change ≥6dB; PWS analysed according to location: foveal (2º), perifoveal (4º),peripheral (remaining 6 to 10º); superior/inferior, temporal/nasal areas
Chen et al.,2008 [212]	Pilot study Autologous RPE-choroid graft subfoveally	Macular dystrophy	MP-1 Mesopic Custom grid (postop grid to include graft & surrounding area) 4-2 staircase	Primary Safety, presence of retinal function Secondary PWS Maximal point sensitivity FAF BCVA Reading acuity CS
Park et al., 2015 [213] NCT01736059	Phase I Intravitreal autologous CD34+ bone marrow stem cells	Ischaemic & degenerative retinal conditions (RVO, AMD, STGD, RP)	MAIA Mesopic Standard 10º (37 points)	Primary AEs, no. of CD34+ cells isolated & injected Secondary MP – MS & % reduced sensitivity

				Changes in Goldmann perimetry, FFA, ERG, OCT, AO-OCT

Table 5: Summary of interventional studies in Inherited retinal disease and their endpoints. Unless otherwise stated, test parameters involved stimuli of Goldmann III size and 200 ms duration. AAV: recombinant adeno-associated virus (vector used for gene therapy) AAV2.REP1; AE: Adverse Events; AO-OCT: Adaptive optics Optical Coherence Topography; CNV:Choroidal neovascularisation; CS: Contrast sensitivity; DA: Dark adaption; ERG: Electroretinography; FFA: Fundus fluorescein angiogram; FST: Full-field stimulus testing; hESC-RPE: human embryonic stem cell-derived retinal pigment epithelium; HFA: Humphrey Field Analyzer; MD: Mean deviation; mERG: multifocal ERG; Min: Minute(s); NAC: *N*-acetylcysteine; Postop: Postoperative; Pts: patients; PWS: Pointwise sensitivity: QoL: Quality of Life measures/questionnaires; RP: Retinitis pigmentosa; RPE: Retinal Pigment Epithelium; *RPGR* RP: X-linked retinitis pigmentosa secondary to *RPGR* defect; RVO: Retinal vein occlusion; STGD1: Stargardt Disease Type 1; VF: Visual field; VFMA: Visual Field Modelling and Analysis; XLRS: X-linked retinoschisis

Study	Study Design & Interventions	Condition	Microperimetry test parameters	Endpoints
Weigert, G., 2011 [146] NCT00879671	Single center RCT Lutein Placebo	Age related Macular Degeneration. (AREDS stages 2, 3, and 4 with no CNV)	MP-1 Mesopic 41 stimuli 12° grid 4-2-1 staircase 3° red cross fixation target	Primary MPOD Secondary BCVA MS
Huang, Y-M., 2014 [79] NCT10528605	Single center RCT 10mg Lutein 20mg Lutein 10mg Lutein + Zeaxanthin Placebo	AREDS classified Early Age-related Macular Degeneration	MP-1 Mesopic 10 minute DA 41 stimuli 10° grid 4-2-1 staircase 3° red cross fixation target	Primary MPOD Secondary MfERG (assessed at 0 and 48 weeks only) MS (assessed at 48 weeks and 24 months only) 1° MS 3° MS 5° MS
Dow, C.T., 2016[96]	Single center RCT Oral telomerase (TA-65) Placebo	Early Age-related Macular Degeneration	MAIA Mesopic 61 stimuli 10° grid 4-2 staircase 1° red circle fixation target	Primary % reduced threshold points MS
Wu, Z., 2019[150] NCT01790802	Multicenter RCT SNL Sham laser	Intermediate Age-Related Macular Degeneration	MAIA Mesopic 37 stimuli 12° grid	Secondary Time to develop late AMD in none study eye Exploratory Rate of change in: BCVA LLVA MS Drusen volume Night Vision questionnaire Impact of Visual Impairment Questionnaire

Sacu, S., 2008 [147] EudraCT No.: 2005-000776-41	Single center RCT Standard PDT Reduced fluence PDT	Neovascular Age-related macular degeneration	MP-1 Mesopic 41 stimuli 12° grid 4-2-1 staircase 5° cross fixation target	<u>Primary</u> BCVA <u>Secondary</u> CRT MS
Dunavoelgyi, R., 2011 [97] EudraCT No.: 2005-000776-41	Single center RCT Standard PDT Reduced fluence PDT	Neovascular Age-related macular degeneration	MP-1 Mesopic 41 stimuli 12° grid 4-2-1 staircase 5° cross fixation target	<u>Primary</u> MS Relative scotoma size (% of points <10dB) Absolute scotoma size (% of points <0dB) <u>Secondary</u> BCVA FA
Rezar-Dreindl, S., 2017 [148] NCT01162746	Single center RCT RM RM + DEX	Neovascular Age-related Macular Degeneration	MP-1 Mesopic 4-2-1 staircase 3° circle fixation target	<u>Primary</u> Time until RM retreatments Total number of RM retreatments <u>Secondary</u> BCVA CFT MS
Limoli, P.G., 2018 [152]	Single center pilot RCT Suprachoroidal autologous graft Control	Dry Age-related Macular Degeneration	MAIA	BCVA MS

Heier, J. S., 2020[98] NCT02247479 NCT02247531	Subgroup of Phase 3 Multicenter RCT Lampalizumab q4w Lampalizumab q6w Sham	Bilateral Geographic Atrophy	MP1 Mesopic 68 stimuli 10-2 grid 4-2 staircase	<u>Primary</u> GA lesion size on FAF <u>Secondary</u> BCVA LLVA Reading speed MS Number of absolute scotoma points (<0dB) FRI NEIVFQ-25
Rinaldi, M., 2016 [151] NCT01968486	Single center RCT Verteporfin + Standard fluence PDT Verteporfin + Reduced Fluence + RM RM	Myopic Choroidal Neovascularisation	MP-1 Mesopic 45 stimuli 12° grid 4-2 strategy	<u>Primary</u> BCVA CRT <u>Secondary</u> Number of RM retreatments Time to first RM retreatment FA and OCT anatomical changes <u>Exploratory</u> MS
Chew, E.Y., 2019 [149] Effect of Ciliary Neurotrophic Factor on Retinal Neurodegeneration in Patients with Macular Telangiectasia Type 2 NCT01949324	Multicenter RCT CNTF implant surgery Sham surgery	Macular Telangiectasia Type 2	MAIA	<u>Primary</u> EZ disruption on SD OCT at 24 months <u>Secondary</u> EZ disruption on SD OCT at 12 month ETDRS letters MS Monocular reading speed 30-2 Humphrey visual fields <u>Post-hoc</u> Aggregate sensitivity loss

Vujosevic, S., 2010 [80]	Single center RCT SMDL ETDRS laser	Diabetic Macular Oedema	MP-1 Mesopic 5 minute DA 45 stimuli 12° radial grid 4-2-1 staircase 1° red ring fixation target	Primary 4° FMS MS FAF Secondary ETDRS letters CRT
Vujosevic, S., 2015 [81]	Single center pilot RCT Yellow Micropulse laser Infrared Micropulse laser	Diabetic Macular Oedema	MP-1 Mesopic 5 minute DA 45 stimuli Custom 12° grid 4-2 staircase 1° red ring fixation target	Primary: BCVA 4° FMS MS Structural parameters on OCT
Gonzalez, V.H., 2015 [164] NCT00789477	Sub group of multicenter randomized, double-masked Phase 2 study **[166, 214]** Laser 0.5q4 IA 2q4 IAI 2q8 IA 2PRN	Diabetic Macular Edema	MP-1 Mesopic 5 minute DA 29 stimuli 16° grid 4-2-1 strategy 1° red ring fixation target	Primary BCVA at 24 weeks Secondary BCVA at 52 weeks % subjects with 15 letter gain CRT number of laser treatments MS in OCT subfields Central 4° (2° radius) Inner 10° (5° radius) Inner to Outer ring (2° to 8° radius) FS
Comyn, O., 2014 [82] NCT01223612	Single center RCT 2-1 randomization RM ETDRS laser	Diabetic Macular Edema	MP-1 Mesopic 45 stimuli Custom 12° grid	Primary Adverse events BCVA FA Exploratory functional 4° FMS MS Colour contrast thresholds Electrophysiology parameters

Mylonas, G., 2020 [165] NCT00682539	Single center prospective randomised study Bevacizumab Triamcinolone	Diabetic Macular Edema	MP-1 Mesopic 41 stimuli 12° grid 4-2-1 strategy 3° red cross fixation target	<u>Primary</u> BCVA CRT (presented in Kriechbaum, K., 2014 **[167]**) <u>Secondary</u> MS Number of absolute scotoma points (<0dB) Absolute scotoma size (% of absolute scotoma points) Relative scotoma size (% of relative scotoma points (≥1 dB and < 10 dB)
Forte, R., 2011 [215]	Single center RCT Flavonoid supplement Control	Diabetic Cystoid Macular Edema without macular thickening	SD-SLO/OCT Mesopic 8° grid 4-2-1 staircase	BCVA CRT MS FS
Wallsh, J. 2016 [179] NCT01449682	Exploratory single center RCT DEX 4 month regime DEX PRN regime	Macular Edema secondary to Retinal Vein Occlusion	MP-1	<u>Primary</u> Multifocal ERG MS <u>Secondary</u> OCT BCVA
Mackensen, F., 2013 [216] NCT00344253	Single center RCT Interferon Methotrexate	Macular Edema in Uveitis (primary or associated with multiple sclerosis)	MP-1 Mesopic 10° grid	<u>Primary</u> Change in BCVA <u>Secondary</u> CRT Inflammatory activity MS NEIVFQ-25 SF36

van Dijk, E.H.C., 2018 [173] NCT01797861	Multicenter RCT Half dose PDT HSML	Chronic Central Serous Chorioretinopathy	MP-1 and MAIA 'to a standard protocol'**[217]** (Threshold measurements from each device converted to single scale as per Parodi, M.B., 2015) **[63]**	Primary Absence of SRF at 6-8 weeks Secondary Absence of SRF at 7-8 months No of repeat treatments required ETDRS letters MS NEIVFQ-25
van Rijssen, T.J., 2020 [174] NCT01797861	Prospective crossover treatment Multicenter RCT **[173]** Crossover to Half dose PDT Crossover to HSML	Chronic Central Serous Chorioretinopathy	MP-1 and MAIA No other parameters provided**[217]** (Threshold measurements from each device converted to single scale as per Parodi, M.B., 2015) **[63]**	Primary Resolution of SRF Secondary ETDRS letters MS NEIVFQ-25
Dang, Y., 2013 [175]	Single center RCT H. Pylori treatment Placebo	Central Serous Chorioretinopathy (positive for Helicobacter Pylori)	MP-1 Mesopic 33 stimuli 15° grid 4-2-1 staircase	Primary Resolution rate of SRF Secondary BCVA MS
Viana, K.I.S., 2020 [177] Brazilian Clinical Trial Number: RBR-3wmd9s	Single center RCT PPV + Phaco PPV deferred Phaco	Full-Thickness Macular Hole	MAIA Mesopic 37 stimuli 6° grid 4-2 staircase	Primary LogMAR BCVA change from baseline Secondary MS % closure rate

Morescalchi, F., 2020 [83] NCT02361645	Single center prospective, randomized, comparative study Complete ILM peel Foveal-sparing ILM peel 12 month follow up	Macular hole > 250µm	OPKO/OTI Mesopic 28 stimuli Polar 3° to 12° grid	<u>Primary</u> ETDRS letters <u>Secondary</u> CRT FMS (mean of 4 central points) Adverse events
Morescalchi, F 2020 [84] NCT02361645	Single center RCT Foveal-sparing ILM peel Control	Degenerative lamellar hole	OPKO/OTI Mesopic 28 stimuli Polar 3° to 12° grid	<u>Primary</u> ETDRS letters <u>Secondary</u> CRT FMS (mean of 4 central points) Structural endpoints Adverse events
Russo, A., 2019 [85] NCT02361645	Single center prospective, randomized, comparative study Complete ILM peel Foveal-sparing ILM peel	Epiretinal membrane	OPKO/OTI Mesopic 28 stimuli Polar 3° to 12° grid	<u>Primary</u> ETDRS letters <u>Secondary</u> CRT FMS (mean of 4 central points) PMS (mean of peripheral 24 points) Adverse events

Eissa, M.G.A.M., 2018 [86]	Single center prospective interventional randomized comparative study With ILM peeling Without ILM peeling	Macula-off rhegmatogenous retinal detachment	OPKO/OTI Mesopic 56 stimuli 10-2 grid 4-2 staircase	Primary BCVA MS 2° MS (mean of 4 central points) Secondary OCT features
Ripandelli, G., 2015 [87]	Multicenter RCT ILM peel No ILM peel	Idiopathic Macular Pucker	MP-1 Mesopic 5 minute DA 33 stimuli 12° grid 4-2-1 staircase 4° red cross fixation target	Primary 4° MS 12° MS Number of absolute scotoma points Secondary BCVA OCT parameters
Romano, M.R., 2018 [218]	Single center prospective, randomized, comparative study Trypan blue 0.15% + brilliant blue 0.05% + lutein 2% Trypan blue 0.15% + brilliant blue 0.025% + polyethylene glycol 3350 4% Indocyanine green 0.05%	Idiopathic epiretinal membrane	MP-1 Mesopic 5 minutes DA 61 stimuli 10° grid 4-2 strategy 2° red cross fixation target	BCVA MS
Pilotto, E., 2011 [219]	Single center prospective randomized study Standard PDT Bolus PDT	Choroidal hemangioma	MP-1 Mesopic 10° grid centerd on lesion 2° ring fixation target	Primary BCVA defined as Stable (±1 line) Improved (>1 line) Decreased (<1line) MS over treated area defined as: Stable (±2 dB) Improved (>2 dB) Decreased (<2 dB)

Table 6: Summary of randomized interventional studies in acquired retinal disease and their endpoints. Unless otherwise stated, test parameters involved stimuli of Goldmann III size and 200 ms duration. AREDS: Age-Related Eye Disease Study; BCVA: Best Corrected Visual Acuity; CFT: Central Foveal thickness;

CMT: Central Macular thickness; CNFT: Ciliary Neurotrophic Factor; CRT: Central Retinal thickness; DEX: Dexamethasone; DHA: Docosahexaenoic acid; ETDRS: Early treatment of Diabetic Retinopathy Study; EZ: Ellipsoid zone; FA: Fluorescein Angiography; FAF: Fundus Autofluorescence; FMS: Foveal Mean Sensitivity (dB); FRI: Functional Reading Index; FS: Fixation stability; FSP: Foveal Sparing; HSML: High-Density Subthreshold Micropulse Laser; IA: intravitreal aflibercept; ILM: Inner Limiting Membrane; LP: Laser Photocoagulation; MDOP: Macular Pigment Optical Density; MfERG: Multifocal Electroretinogram; MS: Mean Sensitivity (dB); NEIVFQ-25: National Eye Institute Visual Function Questionnaire 25; PDT: Photodynamic Therapy; Phaco: Phacoemulsification; PMS: Perifoveal retinal sensitivity; PPV: Pars Plana Vitrectomy; RCT: Randomised control trial; RM: Ranibizimab; SD-OCT: Spectral Domain Optical Coherence Topography; SMDL: Subthreshold Micropulse Diode Laser; SNL: Subthreshold Nanosecond Laser; SRF: Subretinal Fluid