Quantitative physiological measurements to evaluate the response of antivascular endothelial growth factor treatment in patients with neovascular diseases

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Antivascular endothelial growth factor (VEGF) treatment is still used intravitreally worldwide for various neovascular diseases, despite other available, approved treatments. We performed a systematic search of the literature focused on visual physiology studies. We used the online biomedical search engine PubMed and searched key words including “M-chart,” “Preferential Hyperacuity Perimetry,” “microperimetry,” (MP) "electroretinography," and “contrast sensitivity” to estimate treatment efficacy of anti-VEGF treatments in a quantitative manner. Many studies were identified which used a variety of methodologies, disease entities, injected agents, and patient populations, making it difficult to obtain a direct comparison of their results. However, favorable functional outcomes achieved using current quantitative methods would lend further confidence to the effectiveness of a treat-and-extend protocol using intravitreal anti-VEGF for the management of patients with neovascular diseases. Despite anti-VEGF’s wide use, a well-designed longitudinal multicenter study to systematically evaluate and compare different physiological methods or parameters in patients with neovascular diseases is still lacking, though it would benefit therapeutic decisions.

Key words: Antivascular endothelial growth factor treatment, microperimetry and contrast sensitivity, quantitative analysis and electroretinography

Angiogenesis is the process of formation of new capillaries from preexisting blood vessels, which is involved in many pathologic states associated with the formation of new blood vessels.[11] Many pathologic ocular conditions result in vision loss due primarily to angiogenesis, including choroidal neovascularization (CNV) in age-related macular disease (AMD), retinal neovascularization in diabetic retinopathy (DMR), retinal vein occlusion (RVO), and retinopathy of prematurity (ROP).[2-5] As early as 1948, Michaelson[6] hypothesized that an increasing concentration of a diffusible molecule in the retina, which he referred to as “factor X,” was the primary cause of abnormal blood vessel growth in the eye. Early preclinical and clinical studies have since identified vascular endothelial growth factor (VEGF) as a major factor in the principal mechanism behind neovascularization.[7-17] Although the pathogenesis of neovascular disease is not fully understood, inhibition of VEGF has been shown to be an effective method for anatomic and functional outcomes in patients with neovascularization.

To assess the response of anti-VEGF treatment, it is necessary to define it in terms of measurable, reproducible, clinically relevant items.[18] The most visible clinical manifestation of retinal diseases is deterioration of vision, and visual acuity (VA) measurement is simple to implement in a clinical setting. Therefore, recording changes in VA are still regarded by most retinal specialists as mandatory during initial and follow-up assessments and are a standard outcome to correlate with morphological results from different types of devices.[19,20] Neovascular retinal diseases affect VA to a varying degree, and the current treatment of choice, intravitreal injection of anti-VEGF, has proven its visual efficacy in several large randomized clinical trials.[21-30]

Although it is generally recognized that higher best-corrected VA (BCVA) is correlated with better vision, BCVA does not indicate exclusive information regarding retinal function when changes in VA are minimal and is often within the range of variability of the test.[20,31] Distance VA is a reflection of resolution at the foveola, representing acuity in the central 1° of the visual field (VF).[32] Moreover, VA has shown poor correlation with both qualitative and quantitative imaging assessments of macular morphology.[33] Based on clinical experience, morphologic deterioration does not immediately affect VA. Indeed, brain mechanisms can compensate for retinal malfunction and often delay the typical symptoms of neovascularization recurrence until the lesion is relatively large and subfoveal.[34] The availability of an objective and easily performed functional test that correlates well with macular morphology and has a capacity to evaluate the response of
not only the fovea but also the larger macular area would markedly change current clinical practice, allowing for fewer monitoring visits. Various types of functional assays have been compared in terms of their diagnostic capacity, reproducibility, and clinical applicability in identifying the tests with the greatest potential to evaluate the efficacy of a given therapy.\(^\text{[37]}\)

In this review, we address quantitative tests that cover a wide array of functional assessments in response to anti-VEGF therapy, beyond measurement of VA. Evaluation with a different transformation of visual function parameters would allow more information to be gathered for an early response and might act as a more effective method of disease monitoring.

**Methods**

To ensure accuracy, this literature review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement.\(^\text{[39]}\)

A comprehensive search of the literature was conducted for papers using the online biomedical search engine PubMed, Embase, and the Cochrane library. The following terms were used for the searches: neovascular retinal diseases AND (anti-VEGF treatment OR anti-VEGF therapy) AND (M chart OR preferential hyperacuity perimetry (PHP) OR electroretinography (ERG) OR microperimetry (MP) OR contrast sensitivity [CS]). The two authors (IHH and SPP) identified articles using anti-VEGF agents in the management of retinal diseases with varying pathophysiologies that share a common final pathway of pathologic neovascularization. Anti-VEGF therapy was administered either as stand-alone therapy or combined with other interventions, without consideration of injection dose or frequency. All relevant articles were included in this review. Limits for our literature search filters included papers published in English between 1987 and March 2017, including human studies published as randomized controlled trials and nonrandomized comparative studies (cohort or retrospective studies or case–control series). Anti-VEGF uses in ROP and animal studies, editorials, review articles, letters to the editor, and meeting abstracts were excluded from this review.

**Results and Discussion**

A total of 219 articles were initially identified from a literature search. Of these, 46 studies were selected for review after checking for inclusion and exclusion criteria. The studies are summarized in Tables 1-4.

**Quantification of metamorphopsia**

Patients with advanced macular disease, particularly with neovascular AMD and DME, often complain of metamorphopsia, a phenomenon defined as an observed deformation of straight lines due to the displacement of photoreceptors. As metamorphopsia significantly correlates with a patient’s vision-related quality of life, many tests have been introduced to evaluate the distorted vision.\(^\text{[39]}\)

The Amsler grid, introduced in 1947, consists of evenly spaced horizontal and vertical lines and has been commonly used to test for metamorphopsia.\(^\text{[38]}\)

Although the Amsler grid is inexpensive, straightforward, and easily understood by the patient, the results are only descriptive; they are neither precise nor reproducible.\(^\text{[40]}\)

Importantly, the grid can only assess abnormal findings qualitatively; direct quantitative analysis of impairment is not possible. Thus, it is difficult to monitor metamorphopsia over time and to evaluate the efficacy of anti-VEGF treatments.

The M-chart, a diagnostic tool developed by Matsumoto, includes a set of dotted line printouts that can be used to score metamorphopsia according to the minimum dotted line interval needed to null the visual distortion of the patient [Fig. 1].\(^\text{[41,42]}\)

M-chart is a simple, reliable tool to quantify metamorphopsia, and have been adopted in several studies to evaluate response to anti-VEGF treatments [42-44] [Table 1]. Nowomiejska et al.\(^\text{[45]}\) first reported a comparison of M-chart and Amsler grid results with optical coherence tomography (OCT) results in an assessment of metamorphopsia in patients with wet AMD treated with intravitreal bevacizumab injections. Similar assessments were used in patients with DME and RVO.\(^\text{[43,44]}\) They found that the M-score decreased significantly after anti-VEGF treatment in patients with AMD and DME and concluded that treatment efficacy can potentially be assessed by calculating M-score changes in patients with metamorphopsia. However, the M-score did not decrease in patients with RVO, and different results were obtained between the two studies when comparing M-score with BCVA and anatomical results from OCT. A longer follow-up is needed to determine if M-charts can be a complementary test to anatomical imaging and BCVA testing.

An alternative to M-charts is the PHP test, which has recently gained popularity as a device for monitoring visual distortion in patients with CNV.\(^\text{[45,46]}\) PHP uses hyperacuity to detect and quantify the severity of visual defects associated with the development of neovascularization, such as metamorphopsia and scotoma within the central 14° of the visual field. PHP has been used in several studies to quantify metamorphopsia in patients with AMD treated with ranibizumab [Table 1].\(^\text{[37,47,48]}\)

After intravitreal ranibizumab injection, the PHP metamorphopsia test score significantly improved. The clinical observations indicated good correspondence between the results from PHP tests and macular morphology whereas...
changes in BCVA did not significantly correlate with changes in any of the OCT metrics or PHP tests. These findings suggest that PHP tests may be a useful tool to monitor the response to anti-VEGF treatment in a quantitative manner. However, further investigations are needed to establish the sensitivity of PHP compared with other functional and morphological measurements in patients undergoing anti-VEGF treatment for other vitreoretinal neovascular diseases.

Microperimetry
Routine functional measurement of vision, such as Snellen or ETDRS charts, may not reflect local macular (extrafoveal) retinal dysfunction. Perimetry examines the sensitivity of different locations of the retina and reflects retinal morphology. MP, which combines digital fundus imaging with automated perimetry, can quantify the sensitivity of the central retina in a precise fundus-related position, allowing precise quantification of retinal function at specific loci and subsequent correlation with anatomic findings at that locus [Fig. 2]. Furthermore, these noninvasive procedures have an automatic eye tracker that compensates for eye movements during the examination, analyzing macular function even when fixation is unstable.

The value of such evaluations with MP has been previously reported in numerous studies assessing the efficacy and safety of anti-VEGF treatment in eyes with wet AMD, DME, RVO, and myopic CNV. [Table 2].

Useful information on macular function can be obtained by MP parameters, including macular sensitivities, size of dense scotoma, fixation stability, and fixation location. Parallel to VA and anatomic improvement, most studies showed improvement of MP parameters after treatment in spite of the differences in patient characteristics, disease entities, and anti-VEGF therapy. Macular sensitivity increase, absolute scotoma reduction, and stabilization of fixation estimated by MP strongly support evidence of macular function recovery, as suggested by other functional outcomes, after intravitreal anti-VEGF injections for neovascular disease.

However, according to statistical analysis of the correlation with VA and OCT, the results were variable.

Table 1: Studies using quantification of metamorphopsia for the evaluation of anti-vascular endothelial growth factor treatment

| Publication          | Patient n | Type of Disease | Type of injection | Type of test | F/U period | Main results after anti VEGF injection | Notes                  |
|----------------------|-----------|-----------------|-------------------|--------------|------------|----------------------------------------|------------------------|
| Querques et al., 2011 | 14        | wet AMD         | Ranibizumab       | PHP*         | 1 m        | PHP* score ↑                           | Amsler grid (6 patients) |
| Nowomiejska et al., 2013 | 36      | wet AMD         | Bevacizumab       | M charts     | 1 m        | Horizontal M score ↑; Vertical M score ↑ | Visual acuity ↑ (16 patients) |
| Achiron et al., 2015  | 28        | DME RVO         | Not indicated     | M charts     | 1 m        | M score ↑ in DME (71.4%); M score ↑ in AMD (35.7%) | 0% of M score ↑ in RVO |
| Nowacka et al., 2015  | 17        | DME             | Ranibizumab       | M charts     | 6 m        | Metamorphopsia frequency ↑ at 3 mon    | No significant difference at 6 mon |
| Das et al., 2009      | 17        | wet AMD         | Ranibizumab       | PHP*         | 10 days    | PHP* score ↑                           | PHP parameters correlated with SRF changes |
| Querques et al., 2011 | 17        | wet AMD         | Ranibizumab       | PHP*         | 6 m        | PHP* score ↑                           |                                                     |

*Preferential hyperacuity perimeter

Figure 2: A microperimetry grid and the location of the Early Treatment Diabetic Retinopathy Study grid in respect to stimulation loci (left), and an example of a microperimetry examination of a patient with baseline occult choroidal neovascularization (right).
| Publication            | Patient n | Type of Disease | Type of Injection | F/U period | Main results after anti VEGF injection | Notes |
|------------------------|-----------|-----------------|-------------------|------------|---------------------------------------|-------|
| Alexander et al., 2012 | 14        | wet AMD         | Ranibizumab       | 30 wk      | No significant change                 |       |
| Scupola et al., 2010   | 15        | Myopic CNV      | Bevacizumab       | 1 yr       | Mean sensitivity ↑                    |       |
| Munk et al., 2013      | 64        | wet AMD         | Not indicated     | 1 yr       | Mean sensitivity ↑                    |       |
| Parravano et al., 2010 | 18        | wet AMD         | Ranibizumab       | 24 m       | Mean sensitivity ↑                    | Fixation stability ↑ in 9 patients |
| Cho et al., 2013       | 39        | wet AMD         | Ranibizumab       | 12 m       | Mean sensitivity ↑                    | Fixation stability ↑ in 5 patients |
| Bolz et al., 2010      | 29        | wet AMD         | Ranibizumab       | 3 m        | No significant change                 | No significant change in fixation stability |
| Ozdemir et al., 2012   | 21        | wet AMD         | Bevacizumab       | 6 m        | Mean sensitivity ↑                    |       |
| Kriechbaum et al., 2009| 28        | RVO             | Bevacizumab       | 1 yr       | Mean sensitivity ↑                    |       |
| Papadia et al., 2014   | 44        | RVO             | Bevacizumab       | 18 m       | Mean sensitivity ↑                    | Mean defect value from visual field test ↓ |
| Malagola et al., 2006  | 26        | DME             | Bevacizumab       | 24 wk      | Mean sensitivity ↑                    | No significant change in fixation stability |
| Vujosevic et al., 2016 | 49        | DME             | Bevacizumab       | 1 m        | No significant change                 | No significant change in intravitreal dexamethasone treated group |
| Comyn et al., 2014     | 33        | DME             | Ranibizumab       | 48 wk      | Mean sensitivity ↑                    | More improved than laser treated group |
| Yodoi et al., 2008     | 21        | Myopic CNV      | Bevacizumab       | 6 m        | Mean sensitivity ↑                    |       |
| Wang et al., 2012      | 19        | Idiopathic CNV  | Bevacizumab       | 12 m       | Mean sensitivity ↑                    | Fixation stability ↑ in 17 patients |

VA or OCT measurements alone. Among patients with central RVO (CRVO), VF tests were performed to distinguish ischemic from patients without ischemia. Thus, average mean defect values were measured after intravitreal anti-VEGF injections. Although two functional parameters, computerized VFT and MP, were useful and complementary for monitoring evolution, MP was more favorable in the injection group, reflecting extramacular damage (edema).

Electrophysiological tests

Electrophysiological testing of patients undergoing treatment for neovascular diseases may provide additional insight into the safety of a particular treatment beyond that obtained by traditional testing with psychophysical and objective methods. Many studies performed an objective functional evaluation of the patients treated with anti-VEGF agents using several parameters from different electoretinographic tests [Table 3]. As VEGF performs essential physiological functions, total blockage may induce unwanted side effects in the retina. Thus, in spite of the previous clinical success of anti-VEGF agents, safety studies are of utmost importance. As full-field electoretinogram (FERG) responses represent overall retinal function, global deterioration of FERG responses may indicate retinal toxicity. FERG has been established and widely used as a standard test and sensitive tool to detect retinal toxicity after treatment with anti-VEGF agents. The described stability of ERG responses has been underlined by many studies in various neovascular diseases, suggesting that there is at least no significant toxicity at the concentration used routinely in clinical practice. However, a number of studies reported significant improvement after treatment; FERG has been used to assess the damage that might occur when a patient receives intravitreal injections of anti-VEGF agents, not as a general tool to evaluate the efficacy of treatment in most studies.

The macula is only a small part of the retina; changes in macular function would not alter FERG responses. FERG recording in patients with macular abnormalities complicated by neovascular diseases has the disadvantage of masking macular response abnormalities as a “mass retinal response.”

Multifocal ERG (mFERG), developed by Sutter and Tran, is an objective test which reflects the photopic electrical responses to discrete portions of the central of vision and a promising tool for the assessment of retinal function with identification when lesion-associated recordings are identified and analyzed. By rapidly flickering stimuli consisting of black and white hexagons in a pseudorandom m-sequence on a monitor, the retinal responses corresponding to an area of 25°
of the central vision can be mapped. The waveform of mfERG can be considered as a combination of ON and OFF-bipolar cell contributions and smaller contributions from the inner retina and photoreceptors. The P1 and N1 components of the mfERG from several concentric rings centered on the fovea were averaged, and the mean amplitude and mean implicit time of P1 and N1 from each ring summation were analyzed. Neovascularization and related macular diseases generally depress the central peak response density, which is additionally altered in the presence of macular edema. Thus, many studies have described and quantified the electrical activity of the macula using mfERG, and as a result,
Table 4: Studies using contrast sensitivity for evaluation of anti-vascular endothelial growth factor treatment

| Publication                  | Patient n | Type of Disease | Type of Injection | F/U period | Main results after anti-VEGF injection | Notes                                                                 |
|------------------------------|-----------|-----------------|-------------------|------------|----------------------------------------|----------------------------------------------------------------------|
| Munk et al., 2013 [31]       | 64        | wet AMD         | Not indicated     | 1 yr       | Contrast sensitivity ↑                  | No significant change in reading acuity and maximum reading speed     |
| Comyn et al., 2014 [30]      | 33        | DME             | Ranibizumab       | 48 wk      | Contrast sensitivity ↑                  |                                                                      |
| Patel et al., 2011 [33]      | 65        | wet AMD         | Ranibizumab       | 3 m        | No significant change                   |                                                                      |
| Preti et al., 2014 [34]      | 16        | CRVO            | Bevacizumab       | 3 m        | Contrast sensitivity ↑                  |                                                                      |
| Preti et al., 2013 [35]      | 42        | DME             | Bevacizumab       | 6 m        | Contrast sensitivity ↑                  |                                                                      |
| Zuo et al., 2010 [37]        | 12        | PCV             | Bevacizumab       | 6 m        | Contrast sensitivity ↑                  | Combined with PDT treatment                                          |
| Azad et al., 2008 [36]       | 40        | wet AMD         | Bevacizumab       | 6 m        | Contrast sensitivity ↑                  |                                                                      |
| IVAN Study Investigators et al., 2012 [39] | 610 | wet AMD         | Bevacizumab       | 1 yr       | No significant difference between two groups |                                                                      |
| Pece et al., 2011 [38]       | 17        | RVO             | Ranibizumab       | 12 m       | Contrast sensitivity ↑                  | Mean MNREAD time ↑ Reading frequency ↑                               |

Figure 3: Multifocal electroretinography ring topography showing the six concentric rings of 103 hexagons used for analyses (ring 1 and 2 have been summed for the evaluation of macular function)

have also evaluated its function after injection of anti-VEGF agents. [44,60,63,65,67,73-77] The results from studies using mfERG to quantify anti-VEGF treatment are summarized in Table 3. The improvement of P1 and N1 amplitude in the central fovea and lack of deterioration of mfERG wave parameters reported in several studies suggest that the off-label use of anti-VEGF therapy could initiate recovery of cellular function of the retina and is probably nontoxic to the retina.

In pattern electroretinogram (PERG), the P50 wave is partially generated from ganglion cells with significant contribution from retinal neurons distal to the ganglion cell, thus depending on the functional integrity of the macular photoreceptors. The N95 wave is derived in relation to retinal ganglion cell function of the central part of the retina. [76] The results from the analysis of these electrophysiological responses contribute comprehensive information about macular function during anti-VEGF treatment. Based on this theory, several authors have used PERG to evaluate the change of macular function in patients with CRVO, [78] DME, [44,60,79] and neovascular AMD [80] [Table 3]. Although the results of PERG examinations revealed no improvement in patients with DME treated with ranibizumab, Ozkiris [79] showed that an intravitreal injection of 2.5 mg bevacizumab appeared to be effective for treatment of diffuse DME, with an increase in both VA and P50 amplitudes. To additionally investigate the effect of anti-VEGF treatment on the inner retina, the photopic negative response (PhNR) was recorded in patients with CRVO. [78,81] This slow negative potential, which follows the b-wave of the photopic ERG, most likely originates from spiking activity of the retinal ganglion cells and their axons receiving signals from cones. However, Moon et al. [82] and Gardašević et al. [79] reported a significant improvement of PhNR amplitudes after 1 year of treatment. However, direct comparison of the outcomes of the two studies is difficult because their treatment regimens were different.

Most ERG studies that used VA and OCT assessment methods reported an improvement in VA and/or a reduction in central retinal thickness after anti-VEGF treatment. [60,63,65,67,76,77,79,80] However, neither the correlation between VA and ERG results nor the correlation between central retinal thickness and ERG results is clear. There was no significant electrophysiological change accompanied by significant reduction in OCT thickness and/or improvement in VA. [45,60,66,76] While some mfERG studies reported that the mfERG improvement was significant, it was disproportional to the degree of macular thickness. [63,67,69,81] Campa et al. [77] showed that the proportion of patients with a definitive improvement after a course of ranibizumab treatment was twice as high and occurred much earlier when considering mfERG response compared with BCVA, suggesting that mfERG may be more sensitive to improvements in macular function.
Contrast sensitivity

Distance VA has many drawbacks, as it assesses the ability to resolve detail only at high contrast, while real objects have different degrees of variability in contrast and spatial frequency.[83,85] CS testing allows measurement of the patient’s ability to see low contrast patterns and provides additional information on visual function.[84,85] In particular, CS may be a better predictor of performance in tasks requiring distance judgment of real targets, night driving, and mobility than conventional VA. CS is closely linked with both orientation and mobility, and, in patients with macular disease, may be markedly reduced despite near-normal distance VA.[86] The CS of each patient’s affected eye was measured using the commercially available Pelli-Robson chart (Clement Clarke Inc., Harlow, UK), VCTS 6500 chart (Vistech Consultants Incorporation, Dayton, Ohio, USA), Chroma test (City University, London, UK), and Functional Acuity Contrast Test (FACT) chart (Stereo Optical, Inc., Chicago, IL, USA).

Because CS is dominated by retina function, increases in this functional assessment may reflect the recovery of retinal function after treatment.[87] As a result, clinical protocols including CS testing have been used to evaluate the effect of anti-VEGF treatment and showed the variable anti-VEGF treatment strategies lead to improvement in CS in patients with wet AMD,[52,77,83,88,89] ME from RVO,[84,85] and DMR.[90,93] and polypoidal choroidal vasculopathy (PCV).[85] [Table 4] Randomized clinical trials demonstrated that intravitreal anti-VEGF therapy could initiate a larger increase in CS than conventional laser treatment in patients with DMR.[90,93] One-year findings from the IVAN randomized trial revealed that CS did not differ significantly between drugs or treatment regimens in patients with wet AMD.[87] Low spatial frequencies have a crucial role in perception of real targets, and middle spatial frequencies have more influence on orientation and mobility and are not related to VA.[91] Thus, these results suggest that the intravitreal injection of anti-VEGF agents can improve the quality of life for patients with neovascular diseases through an increase in CS.

Conclusion

The present review provides valuable information on various characteristics of physiological biomarkers in neovascular diseases and shows that VA change or OCT alone may not be the optimal parameter(s) for evaluating the efficacy of anti-VEGF treatments. There has been conflicting data regarding the improvement of visual function in patients with neovascularization who were treated with intravitreal anti-VEGF injections. In part, this has been attributed to heterogeneity within a patient cohort in terms of severity, a multitude of confounding factors and underlying etiologies in neovascularization, differences in the frequency of injections and pharmacokinetics between anti-VEGF agents, unreliable evaluation pre- and post-treatment, and the overall variability in measurements of clinical signs. Our review focused on methodological issues, including various neovascular disorders and anti-VEGF agents; we disregarded the anti-VEGF treatment regimen, making it difficult to compare the outcome of a single anti-VEGF agent against a single functional assessment. A lot of novel and unique physiological tests have been available recently, including functional OCT, chromatic contrast, investigating changes of preferred retinal location and vision-related quality of life.[92-95] However, this review has a limitation that we only included quantitative measurement methods, which we considered more accessible in general clinical settings. Nevertheless, our review proved that intravitreal injection of anti-VEGF agents appear safe and may offer a new therapeutic opportunity for neovascular diseases with a significant impact on visual function. Although VA is an important and well-established physiological test to assess the efficacy of anti-VEGF treatments in clinical trials, it does not seem to comprehensively reflect overall visual function gain or improvement in vision-related quality of life. Additional visual function variables, we addressed should be examined in routine clinical practice as well as in multicenter trials to quantify the efficacy of anti-VEGF treatment and correlate with valuable morphological outcomes. It is therefore of great importance to improve our understanding of retinal neovascularization so we can develop more detailed recommendations regarding when and when not to start treatment. Physiological testing will be an objective way, in which to continue this work.

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Conflicts of interest
There are no conflicts of interest.

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