Longitudinal Assessment of Aqueous Cytokines in DME in correlation with OCT and UWFA biomarkers: Bridging the Anatomic-Biologic Pathway with IMAGINE

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Background

- Diabetic macular edema (DME)is the most common cause of visual decline in middle and older individuals.
- The pathogenesis of DME is tied to cytokines originating from hypoxic tissue, subsequently causing endothelial cell dysfunction, a breakdown of the blood-retinal barrier, and increased vascular permeability.
- A critical cytokine implicated in macular edema is vascular endothelial growth factor (VEGF).

Summary

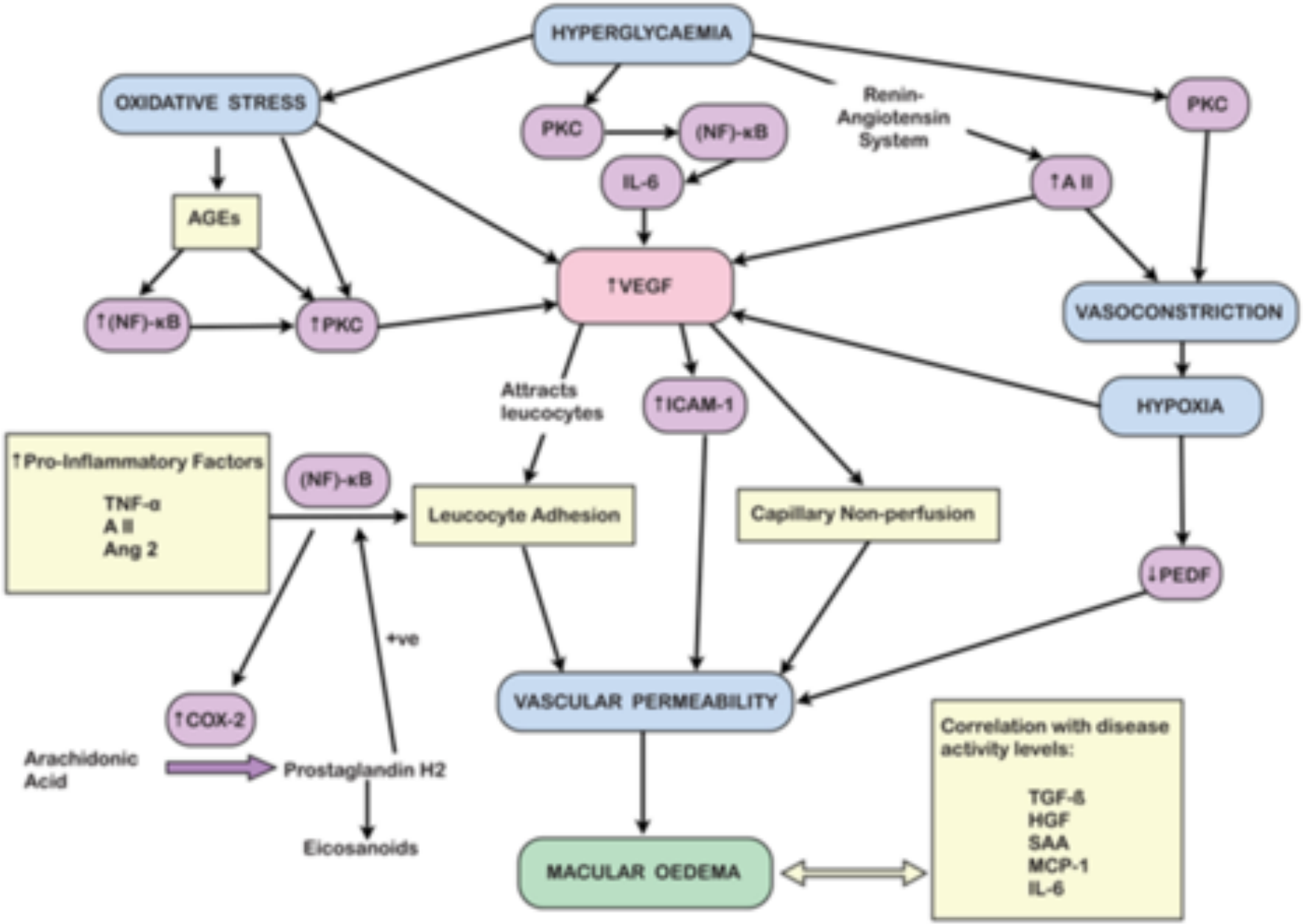
- This study evaluated the longitudinal dynamics of aqueous humor cytokines in eyes undergoing anti-VEGF therapy, as well as the link between cytokine levels and therapeutic response on OCT and UWFA.
- For OCT categorization: LIF, IL-6 and MCP-1 were increased in Nonresponders.
- Thus, the Responders and the Super Responders more so represent eyes with a more VEGF driven phenotype, while the Nonresponders and to a lesser extent the Slow Responders may represent a more multifactorial, inflammation-driven phenotype that would benefit from alternate therapy.

Background

- Despite these promising results, a significant proportion of patients have an incomplete response to anti-VEGF therapy anatomically, functionally or both.
- A possible explanation for this phenomenon is the co-existence of multiple pathways: a predominantly VEGF dependent cycle as well as alternative, inflammatory-based avenue.

Modi A, Sharma K, Sudhakar NP, Yadav NK. Aqueous humor cytokines and therapeutic customization in nonresponding macular edema secondary to retinal vein occlusion. *Retinal cases & brief reports.* 2019.

Zeng Y, Cao D, Yu H, et al. Comprehensive analysis of vitreous chemokines involved in ischemic retinal vein occlusion. *Molecular vision.* 2019;25:756-765.



https://www.eyenews.uk.com/features/ophthalmology/post/the-role-of-inflammation-in-the-pathophysiology-of-dmo

Purpose

- Firstly, this study aims to characterize the longitudinal aqueous humor cytokine profiles in DME patients receiving intravitreal ranibizumab and delineate which baseline cytokines which are predictive of anatomic resolution.
- Secondly, it aims to evaluate the association of higher-order optical coherence tomography (OCT) and ultra-widefield fluorescein angiography (UWFA) features with underlying intraocular cytokine expression and their role in predicting response to anti-VEGF therapy.

Methods

- The IMAGINE study is a post-hoc study evaluating cytokine expression within the aqueous samples and an in-depth assessment of the imaging studies obtained throughout the DAVE study performed by Brown and colleagues.*

*Brown DM, Ou WC, Wong TP, et al. Targeted Retinal Photocoagulation for Diabetic Macular Edema with Peripheral Retinal Nonperfusion: Three-Year Randomized DAVE Trial. *Ophthalmology.* 2018;125(5):683-690.

DAVE

- 3-year prospective randomized trial evaluating ranibizumab alone compared to combination therapy with targeted retinal photocoagulation (TRP) to areas of nonperfusion in treatment-naïve eyes with DME.
- All eyes received 4 doses of monthly 0.3mg ranibizumab injections before starting monthly visits with as needed retreatment based on disease activity (*pro re nata* (PRN)) for the remainder of the study.
- TRP was performed at week 1 in the eyes randomized to that treatment arm to areas of retinal capillary nonperfusion outside the macula with possible retreatment at months 6, 18, and 25.

Methods

- All study eyes with available concurrent aqueous humor samples from baseline were included in the analysis. Aqueous humor samples were obtained when able at baseline, month 3, and month 12 through paracentesis and stored at -80 degrees Celsius.
- Concentrations of the cytokines were measured at each time-point in quadruplicate, averaged, normalized, and values below the limit of detection (LOD) for each cytokine were forced to 0. For this report, only cytokines with a detectable level in at least 20% or more samples were included in analysis.

Cytokines

Activin A Agouti Related Neuropeptide (AgRP) Angiogenin Angiopoietin-2 (ANG-2) Angiopoietin like 4 (ANGPTL4) Basic Fibroblast Growth Factor (bFGF) Epithelial-Neutrophil Activating Peptide (ENA-78) Growth Related Alpha Protein (GRO) Heparin-binding EGF-like Growth Factor (HB-EGF) Hepatocyte Growth Factor (HGF) Interferon Gamma (IFNg) Insulin-like Growth Factor (IGF-1) Interleukin-1a (IL-1a) Interleukin-2 (IL-2) Interleukin-6 (IL-6 Interleukin-8 (IL-8) Interleukin-17 (IL-17)	Interferon Gamma-Induced Protein 10 (IP-10) Leptin Leukemia Inhibitory Factor (LIF) Monocyte Chemotactic Protein-1 (MCP-1) Platelet Derived Growth Factor Subunit B (PDGF-BB) Phosphatidylinositol Glycan Anchor Biosynthesis Class F (PIGF) C-C Motif Chemokine Ligand 5 (RANTES) Transforming Growth Factor Beta 1 (TGFb1) Tissue Inhibitor Of Metalloproteinases 1 (TIMP-1) Tissue Inhibitor Of Metalloproteinases 2 (TIMP-2) Angiopoietin-1 (ANG-1) Angiostatin C-X-C Motif Chemokine Ligand 16 (CXCL16) Epidermal Growth Factor (EGF)	Fibroblast Growth Factor-4 (FGF-4) Follistatin Granulocyte Colony Stimulating Factor (G-CSF) Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) C-C Motif Chemokine Ligand 1 (I-309) Interleukin-1b (IL-1b) Interleukin-4 (IL-4) Interleukin-10 (IL-10) Interleukin-12p40(IL-12p40) Interleukin-12 p70 (IL-12p70) Interferon-inducible T cell a-Chemoattractant (I-TAC) Monocyte Chemotactic Protein-2 (MCP-2) Monocyte Chemotactic Protein-3 (MCP-3)) Monocyte Chemotactic Protein-4 (MCP-4)	Matrix Metallopeptidase 1 (MMP-1) Matrix Metallopeptidase 9 (MMP-9) Platelet And Endothelial Cell Adhesion Molecule 1 (PECAM-1), Transforming Growth Factor Alpha (TGFa) Transforming Growth Factor Beta 3 (TGFb3) Tyrosine Kinase With Immunoglobulin Like And EGF Like Domains 1 (Tie-1) Tyrosine Kinase With Immunoglobulin Like And EGF Like Domains 2 (Tie-2) Plasminogen Activator Urokinase Receptor (uPAR) VEGF-A

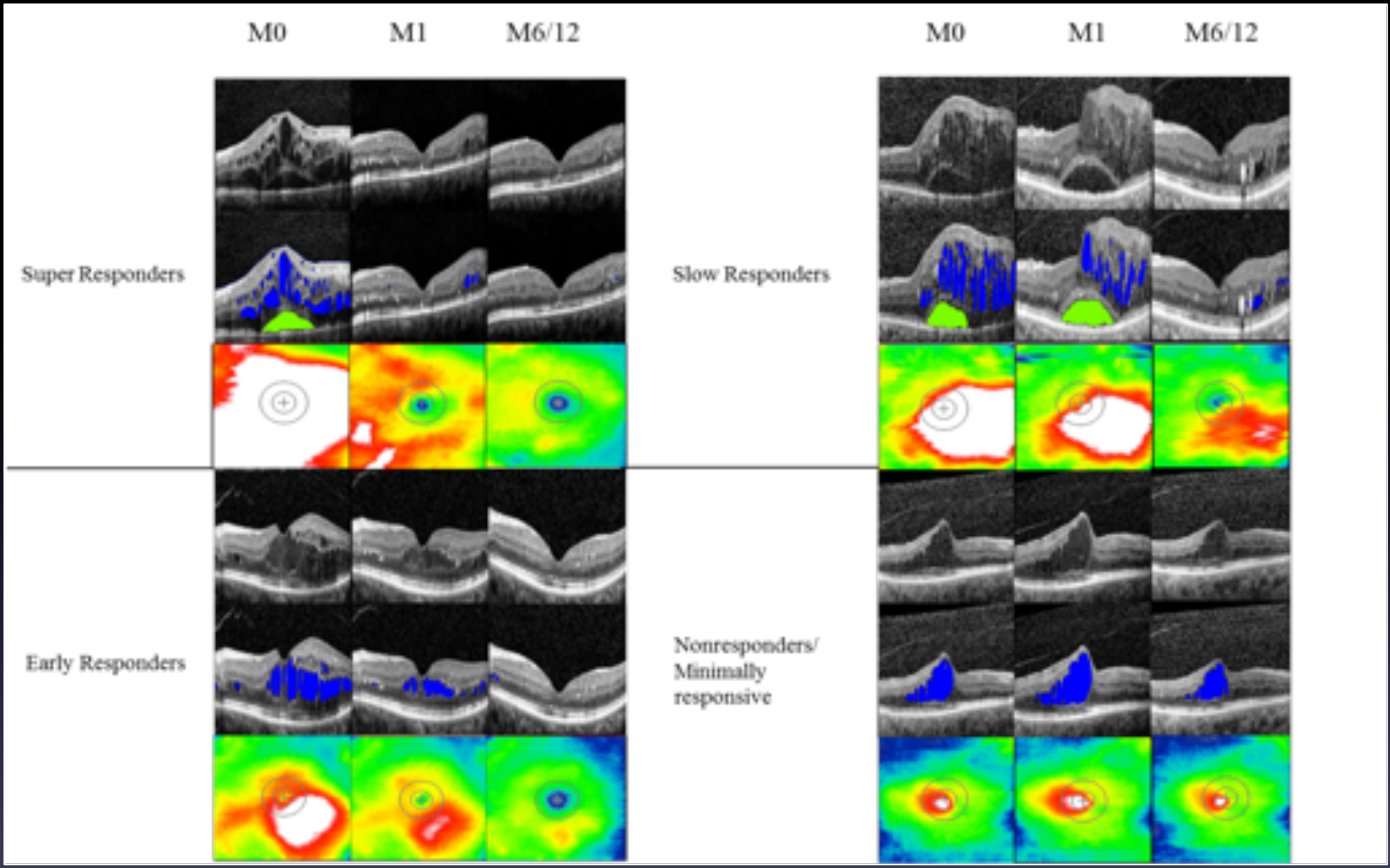
Higher Order OCT Analysis

- Spectral domain-OCT (SD-OCT) macular cube scans were imported into previously described software.
- The macular OCT scans underwent an automated analysis to generate intraretinal and subretinal fluid extraction metrics as well as segmentation of the internal limiting membrane (ILM), ellipsoid zone (EZ) and retinal pigment epithelium (RPE). These delineations were used to calculate thickness, area and volume measurements of various retinal sections. Automated segmentation was reviewed for each B–scan by two concurrent trained readers. Using a previously described software platform, SD-OCT macular cube scans were segmented to determine intraretinal fluid volume, as well as central subfield thickness.

Ehlers JP, Uchida A, Hu M, et al. Higher-Order Assessment of OCT in Diabetic Macular Edema from the VISTA Study: Ellipsoid Zone Dynamics and the Retinal Fluid Index. *Ophthalmology Retina.* 2019;3(12):1056-1066.

Higher Order OCT Analysis

- Overall retinal thickness was assessed (internal limiting membrane to the retinal pigment epithelium (RPE)) as well as from the ellipsoid zone (EZ) to RPE (i.e., surrogate for ellipsoid zone integrity/photoreceptor outer segment length).
- *En face* EZ-RPE topographic thickness maps were generated to visualize EZ changes such as total attenuation (EZ-RPE thickness = 0 micron) or partial attenuation (EZ-RPE thickness < 20 microns).
- Measurements were taken over panmacular scans, as well as the central macular zone (1 mm radius from the fovea) and central subfield (0.5 mm radius) from the fovea.
- Fluid feature extraction was also performed to provide intraretinal fluid and subretinal fluid volumes.
- Retinal fluid index (RFI) was defined as: RFI = 100 × IRF volume / (total retinal volume - SRF volume) The RFI is a percentage which denotes the relative amount of IRF volume against retinal volume in a designated area A percentage of 0% represents no IRF, while 100% represents total IRF.



Representative optical coherence tomography foveal B-scans from Responder categories without volumetric fluid segmentation (first row), with fluid segmentation (second row), and ILM-RPE thickness map for each category taken at baseline, month 1, and month 6 or 12. Color on B-scan distinguishes intraretinal (blue) and subretinal (green) fluid.

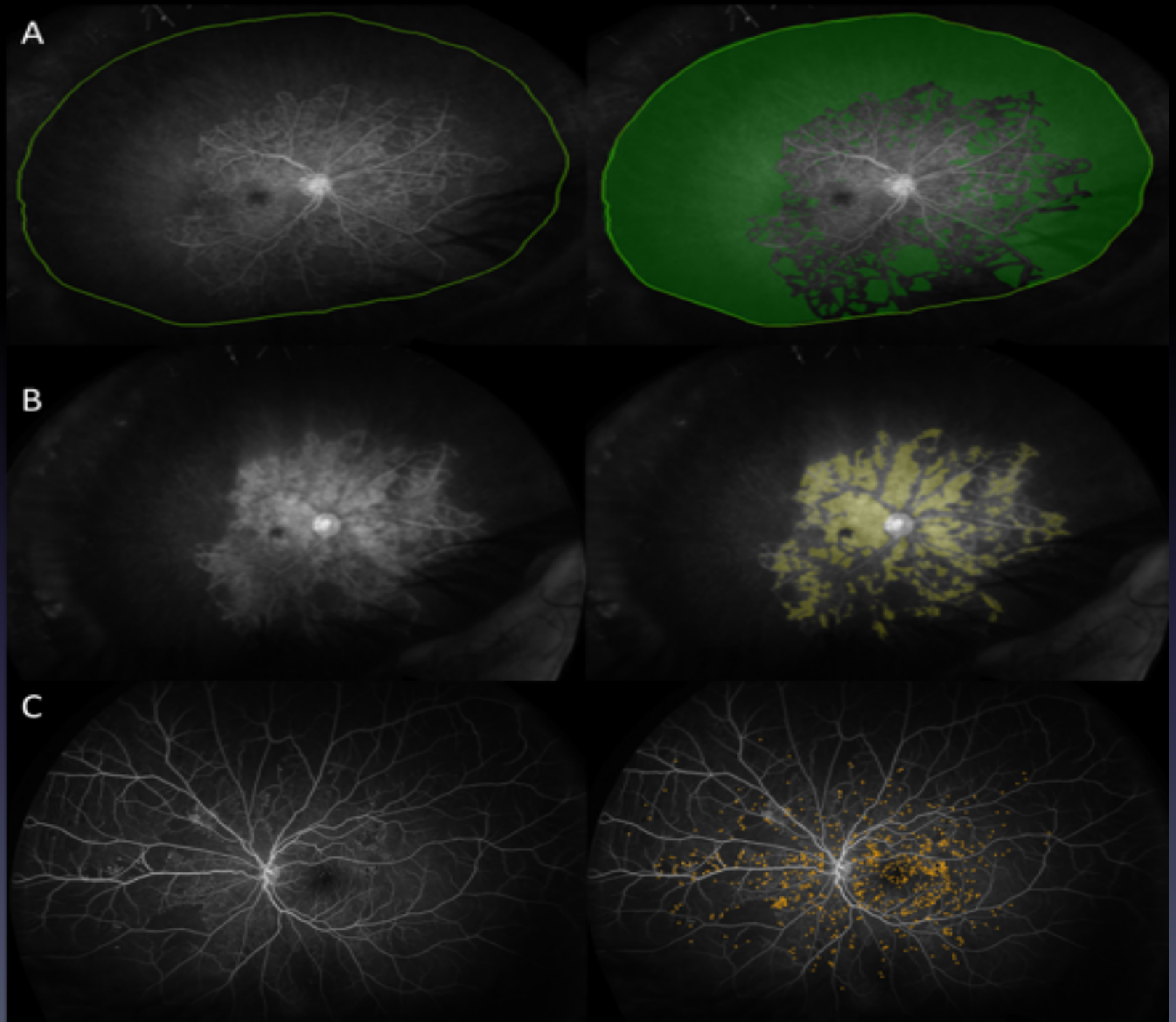
Categorization of Eyes Based on Response

- Based on OCT profiles, four categories of response patterns were formed.
- Super Responders:
  - Defined as ≥80% reduction of the baseline IRF volume, SRF volume <0.001mm^3 and/or a ≥80% or decrease in excess thickening of the central subfield thickness (CST). Excess thickening was defined as CST beyond 300µm. This response must have been obtained within 1 injection.
- Early responders:
  - If the above parameters were met within 3 injections.
- Slow responders:
  - If the parameters described in #1 were met between month 3 and 12.
- Non-Responders/Minimal Responders:
  - If 50% or more of the initial IRF or CST was maintained by month 12.

Ultra Wide Field FA Analysis

- Preceding UWFA image analysis, a previously reported dewarping transformation tool processed all scans creating the projected retinal image.
- Early images were analyzed for ischemia and microanerysym assessment.
- Late images underwent leakage assessment initially by an automated software platform
- Ischemia, or nonperfusion, was defined as areas of hypofluorescence in early-phase imaging.
- Leakage was defined as areas of hyperfluorescence in late-phase imaging.
- Microaneurysms were defined as circular hyperreflective foci marked on early-phase imaging.
- This image analysis was performed at baseline, month 3, and month 12.

Figueiredo N, Srivastava SK, Singh RP, et al. Longitudinal Panretinal Leakage and Ischemic Indices in Retinal Vascular Disease after Aflibercept Therapy: The PERMEATE Study. *Ophthalmol Retina.* 2020;4(2):154-163.


A
B
C

Categorization of Eyes Based on Response

- Eyes were classified into multiple UWFA Responder groups based on longitudinal response between baseline and month 12 for panretinal leakage and panretinal MA count.
- MA Responders:
  - 25% or more reduction in panretinal MA count at any time over 12 months of treatment
- MA Nonresponders:
  - Did not meet the 25% reduction threshold by 12 months.
- Leakage Responders:
  - 50% or more reduction in panretinal leakage by 12 months
- Leakage Nonresponders:
  - Less than a 25% reduction in leakage in the same time course.
- Eyes with leakage between 25% and 50% were considered indeterminate and not included in leakage response analysis.

Results: Baseline Clinical Characteristics and

Parameter	*All (n=24)*	*Responders (n = 19)*	*Nonresponders (n = 5)*
Age (y), mean (SD)	54.6 (9.1)	51.5 (8.5)	66.6 (5.7)
Male sex, n (%)	17 (85.0)	17 (89.5)	2 (40.0)
Right eye, n (%)	10 (50%)	7 (36.8)	3 (60.0)
Hemoglobin A1c (%), mean (SD)	8.2 (2.2)	8.4 (2.1)	7.3 (2.5)
ETDRS BCVA, mean (SD)	58 (13.0)	60 (11.5)	50 (16.3)
Severity of retinopathy, n (%)			
Mild NPDR	1 (4.2)	0 (0)	1 (20)
Moderate NPDR	3 (12.5)	3 (15.8)	0 (0)
Severe NPDR	12 (50.0)	10 (52.6)	2 (40)
PDR	8 (3.3)	6 (31.5)	2 (40)

Cytokine Analysis: Longitudinal Changes

- Of the 54 cytokines evaluated, 27 had levels above detection threshold across all visits in both DAVE and WAVE
- Across all eyes, VEGF concentrations were significantly lower at month 3 ($n=16$, $p<0.001$) and month 12 ($n=15$, $p<0.001$) following initiation of treatment.
- MMP-1 increased at 12 months compared to baseline following treatment ($p=0.036$)
- IP-10 trended toward a significant elevation at month 12 ($p=0.065$) and MCP-1 trended towards decreased levels ($p=0.073$).

Cytokine Analysis: Responder Categories

Cytokine Mean pg/mL (SD)1	Super Responders	Super & Early Responders	All Responders	Nonresponders
AgRP	2.07 (2.72)	4.05 (4.83)	3.41 (4.41)	4.53 (1.88)
ANG-1	43.15 (66.64)	47.81 (69.45)	35.35 (60.72)	41.72 (43.52)
ANGPTL4	1222.45 (1598.96)	1677.42 (1857.56)	1433.65 (1768.18)	303.72 (294.13)
IL-6	15.31 (24.1)	32.58 (56.73)	23.83 (48.58)	0 (0)
IP-10	3.99 (6.95)	8.74 (9.36)	9.95 (13.02)	9 (6.42)
Leptin	5.41 (16.23)	17.7 (30.43)	17.13 (27.12)	107.43 (187.4)
LIF	29.01 (38.25)	28.92 (35.6)	25.34 (33.36)	23.08 (31.67)
MCP-1	513.34 (780.09)*	486.36 (688.26)*	406.73 (578.1)*	809.5 (390.77)
MCP-4	3.38 (3.69)	2.93 (3.4)	2.64 (3.17)	1.52 (2.08)
MMP-1	45.06 (135.17)	31.19 (112.47)	32.62 (102.77)	0 (0)
MMP-9	73.34 (108.13)	59.94 (91.26)	94.89 (172.02)	99.21 (119.51)
PECAM-1	2998.75 (2457.4)	2434.26 (2292.33)	2347.66 (2114.88)	2805.84 (1736.81)
TGFa	0.05 (0.05)	0.06 (0.07)	0.05 (0.06)	0.04 (0.04)
uPAR	358.84 (199.24)	410.24 (224.67)	360.95 (219.94)	336.25 (110.34)
VEGF	880.01 (694.98)*	848.23 (612.16)*	732.58 (609.93)	245.43 (124.75)

1 Standard deviation (SD)

OCT Imaging Biomarkers and Cytokines

Baseline Aqueous Humor Cytokines with Retinal thickness, Intraretinal and Subretinal Fluid Parameters

ILM-RPE Volume minus SRF (mm^3)	**16.0 (4.2)**	**15.0 (3.4)**	10.9 (1.6)
IRF CSV (mm^3)	0.16 (0.16)	0.12 (0.12)	0.036 (0.053)
IRF Volume mm^3	**2.0 (1.7)**	**1.6 (1.3)**	0.44 (0.37)
ILM-RPE CSMT plus SRF µm	**613.7 (224.1)**	**557.9 (178.3)**	359.5 (65.3)
ILM-RPE Volume plus SRF mm^3	**16.1 (4.3)**	**15.2 (3.5)**	10.9 (1.6)
Central Macular IRF Index	0.33 (0.47)	0.24 (0.33)	0.084 (0.062)
Central Macular SRF Index	0.067 (0.10)	0.062 (0.10)	0.0006 (0.001)
Central Macular TRF Index	0.28 (0.15)	**0.30 (0.33)**	0.085 (0.063)
Central Subfield IRF Index	0.33 (0.20)	0.28 (0.21)	0.11 (0.146)
Central Subfield TRF Index	0.43 (0.23)	**0.37 (0.24)**	0.12 (0.14)
Macular IRF Index	**0.11 (0.08)**	**0.098 (0.06)**	0.037 (0.03)
Macular SRF Index	0.009 (0.01)	0.008 (0.01)	0.00007 (0.0002)
Macular TRF Index	**0.12 (0.08)**	**0.11 (0.063)**	0.037 (0.028)

ILM: Internal limiting membrane, RPE: Retinal Pigment Epithelium, CST: Central Subfield Thickness, SRF: Subretinal Fluid, IRF: Intraretinal Fluid, CSV: Central Subfield Volume, EZ: Ellipsoid Zone, CSMT: Central Subfield Mean Thickness, TRF: Total Retinal Fluid.

Baseline Aqueous Humor Cytokines with Outer Retinal Integrity

- Multiple cytokines correlated with panmacular EZ attenuation, including ANGPTL, bFGF, FGF-4, LIF, and HGF.
- In addition, increased central subfield EZ-RPE volume correlated with decreased CXCL16, HGF, and VEGF-A.
- Panmacular EZ-RPE volume similarly correlated with decreased ANGPTL4, uPAR, and CXCL16.

Correlation of Aqueous Humor Cytokines with UWFA Imaging Biomarkers in Treatment-Naïve DME: UWFA Parameters Correlations

Cytokine	UWFA feature	Correlation Coefficient	P-value
ANGPTL4	Panretinal Leakage	0.769080911	4.61E-05
ANGPTL4	Peripheral Leakage	0.693082206	0.0004955
VEGF	Panretinal Leakage	0.702597403	0.0005487
AgRP	Macular Ischemia	0.685398088	0.0008522
IL-12p40	Macular Leakage	0.657142857	0.0015834
IL-6	Panretinal Leakage	0.642147583	0.0016979
uPAR	Peripheral Leakage	0.649350649	0.0018651
uPAR	Panretinal Leakage	0.622077922	0.0032004
MCP-4	MA Total Count	0.596172034	0.004341
IL-6	Peripheral Leakage	0.583059578	0.0055348
PECAM-1	MA Total Count	0.579667134	0.0058844
PECAM-1	Peripheral MA Count	0.577663676	0.0060993
MCP-4	Peripheral MA Count	0.568116879	0.0072142
VEGF	Peripheral Leakage	0.564935065	0.0086393
IL-12p40	Macular Ischemia	0.556230375	0.0108695
ANGPTL4	Macular Ischemia	0.542471987	0.0134661
IL-12p70	Macular Leakage	0.514450602	0.0170298
uPAR	Macular Ischemia	0.517739535	0.0193775
TIMP-1	Macular Ischemia	0.516984813	0.0195859
Leptin	Macular Ischemia	0.491550917	0.0277233
IL-12p40	Macular MA Count	0.475479077	0.0293704
TIMP-1	Panretinal Ischemia	0.485714286	0.0314755

Cytokine	UWFA feature	Correlation Coefficient	P-value
HGF	Peripheral Leakage	0.471428571	0.0324122
MCP-4	Macular MA Count	0.467270059	0.0327
TIMP-2	Macular Ischemia	0.476984528	0.03346
CXCL16	Peripheral Leakage	0.466233766	0.0345727
PECAM-1	Macular MA Count	0.435559608	0.0484293
HGF	Macular Ischemia	0.441512578	0.051313
VEGF	Panretinal Ischemia	0.442105263	0.0524142
I-309	MA Total Count	0.42801448	0.052913
CXCL16	Macular Ischemia	0.432455909	0.0568639
VEGF	Peripheral Ischemia	0.433082707	0.057859
MCP-1	Macular Ischemia	0.420380352	0.0649604
I-309	Macular MA Count	0.407340496	0.0668341
TIMP-1	Panretinal Leakage	0.407792208	0.0676536
Leptin	Peripheral Leakage	0.405854135	0.0679322
HGF	Panretinal Leakage	0.406493506	0.0685968
IP-10	Macular Ischemia	0.414868629	0.0689321
TIMP-1	Peripheral Leakage	0.403896104	0.0705137
TIMP-1	MA Total Count	-0.402597403	0.0714875
TIMP-1	Peripheral Ischemia	0.409022556	0.0745263
ANG-1	Macular Leakage	0.396980437	0.0747736
Angiogenin	Peripheral Ischemia	0.401503759	0.0804202
Angiogenin	MA Total Count	-0.38961039	0.0818011
MMP-1	Panretinal Ischemia	-0.39687457	0.0831712
TIMP-1	Macular MA Count	-0.385190017	0.0846468
LIF	Macular Ischemia	0.393554389	0.0860199

Cytokine Analysis: Responder Categories

Cytokine	UWFA Feature Responder Group	Responder Group Cytokine Mean Concentration (SD)	Comparison Group Cytokine Mean Concentration (SD)	P-value
VEGF	MA Count	332.58 (240.67)	846.59 (439.38)	**0.006**
TIMP.2	Panretinal Leakage	12063.38 (3868.54)	7062.96 (2856.22)	**0.037**
uPAR	MA Count	285.21 (148.04)	469.49 (222.69)	**0.043**
AgRP	Panretinal Leakage	4.69 (4.46)	0.61 (1.22)	**0.046**
IP.10	MA Count	6.66 (6.08)	16.06 (15.13)	0.067
ANGPTL4	MA Count	834.92 (1496.22)	1786.96 (1885.1)	0.072
HGF	MA Count	187.13 (112.31)	347.94 (208.72)	0.099
IL.6	MA Count	3 (9.97)	37.68 (63.36)	0.099

Conclusions

- This study evaluated the longitudinal dynamics of aqueous humor cytokines in eyes undergoing anti-VEGF therapy, as well as the link between cytokine levels and therapeutic response on OCT and UWFA.
- For OCT categorization: LIF, IL-6 and MCP-1 were increased in Nonresponders.
- Thus, the Responders and the Super Responders more so represent eyes with a more VEGF driven phenotype, while the Nonresponders and to a lesser extent the Slow Responders may represent a more multifactorial, inflammation-driven phenotype that would benefit from alternate therapy.

- To the best of our knowledge, no study has correlated outer retina integrity parameters with intraocular cytokines.
- Several cytokines correlated with increased disruption to EZ integrity including VEGF-A, ANGPTL4, LIF, and HGF.

- Leakage parameters correlated strongly with several cytokines including VEGF, ANGPTL4, and IL-6.
- Ischemia parameters correlated with several including increased TIMP-1 and ANGPTL4.
- Increased microaneurysm count correlated with MCP-4 and PECAM-1.
- These cytokine profiles may provide an avenue for therapeutic targets aside from the VEGF pathway

Thank You

- Justis P. Ehlers, MD