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Assessing the Impact of Pharmacological Pupil Dilation on Anterior Chamber Cells Counting Using OCT

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Purpose: Anterior chamber (AC) cells are seen as hyperreflective dots (HRDs) on anterior segment OCT (AS-OCT), but many factors can affect their objective counting. The aim of this study was to evaluate the effect of pharmacological dilation with tropicamide 0.5% (T) or phenylephrine 2.5% + tropicamide 0.5% (PH + T) on the assessment of AC HRDs density measured in healthy eyes by AS-OCT.

Design: Cross-sectional, observational study.

Participants: Healthy subjects with no history of uveitis, no ocular or systemic conditions, and no recent ocular surgery.

Methods: Participants underwent comprehensive ophthalmic examinations, including single-line AS-OCT (16.5 mm; μm:pixel ratio = 8.79; Anterion, Heidelberg Engineering) imaging before (miosis) and after pupil dilation with either T or PH + T drops. Hyperreflective dots were quantified using FIJI (National Institutes of Health, Bethesda) through the Analyze Particles tool after image binarization. Hyperreflective dot density was calculated by dividing the total HRDs by the AC area and expressed as the number per 10 000 pixels^2. Iris displacement after dilation was measured using the apex-to-pupil distance (APD).

Main Outcome Measures: Anterior chamber HRDs density before and after dilation measured by AS-OCT. A generalized Poisson mixed-effects model was used to evaluate the effects of group (miosis, T, PH + T), lens status (phakic, pseudophakic), and their interaction on HRD density. Covariates included age, sex, iris color, and variation in APD after dilation (APDD).

Results: A total of 100 eyes from 59 healthy participants (55.9% female), mean age 73.0 ± 11.7 years were included. Mean (standard deviation) HRDs density (per 10 000 pixels^2) significantly increased from 0.79 (1.44) in normal photopic conditions to 1.71 (3.30) after the dilatation in the T group and from 0.52 (0.83) to 15.8 (25.8) in the PH + T group ($P < 0.001$). Dilation with T ($P = 0.03$) or PH + T ($P < 0.001$), increased APDD ($P < 0.001$), and the interaction between PH + T and phakic status ($P < 0.001$) were all significantly associated with higher HRDs density.

Conclusions: Pharmacological dilation, especially with PH + T, significantly increases HRDs in the AC of healthy subjects, with a more pronounced effect in phakic eyes and greater APDD. These findings suggest increased iris-lens friction and potential pigment release after dilation, providing critical considerations for standardizing AS-OCT-based assessments of AC inflammation.

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Evaluating the content of the anterior chamber (AC) is critical for the management of intraocular inflammatory disorders and is typically carried out using slit lamp biomicroscopy. In particular, accurate identification and quantification of cells and proteins in the AC are essential for assessing the severity of intraocular inflammation (IOI) and evaluating treatment responses in uveitis.[1] To standardize this assessment, the Standardization of Uveitis Nomenclature Working Group proposed an ordinal scale ranging from 0 to 4, based on the number of cells detected within a 1 mm × 1 mm light beam during slit lamp examination, as the gold standard for measuring AC inflammation.[2]

Despite its practicality, this method is frequently criticized for its lack of objectivity due to significant inter- and intraobserver variability in IOI grading across both clinical and research settings.[3] Specifically, Kempen et al reported that the highest agreement is observed at the scale's

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extremes (0 or 4), with diminishing consistency as scale variations of 1° occur. Additionally, the use of an ordinal rather than a continuous scale inevitably decreases the sensitivity in detecting treatment responses, even following appropriate therapy.[3]

To address these limitations, the laser flare photometer has emerged as a reliable and quantitative method for measuring cells and protein in the aqueous humor.[4–6] However, its high costs and limited consensus on its clinical applications have significantly restricted its widespread adoption.[7] More recently, anterior segment OCT (AS-OCT) has gained traction as an imaging modality for identifying inflammatory cells in the AC, which appear as hyperreflective dots (HRDs) against the hyporeflective background of the aqueous humor.[8–11] While initial results from these studies are promising, further investigations are needed to refine the measurements, standardize acquisition protocols, and enhance the reliability of AS-OCT–based AC cells counting.

One of the unresolved questions is the impact of pupil dilation on OCT-based measurements of AC cells. Pharmacological dilation, for instance, can cause pigment dispersion in the aqueous humor,[12] producing HRDs on AS-OCT that are hardly distinguishable from inflammatory cells.[11] By contrast, previous studies suggest that mydriatic agents may reduce the levels of aqueous proteins and cells in healthy individuals,[13,14] potentially leading to either underestimation or overestimation of IOI severity when using AS-OCT.

The aim of the current study was to assess the effect of pharmacological dilation, obtained with 2 different mydriatic agents, on the density of HRDs observed with AS-OCT in the AC of healthy subjects, and consequently affecting the IOI assessment through this technique.

Methods

Study Population

This study was conducted between March 2024 and June 2024 at the Eye Clinic of Ospedale Luigi Sacco Hospital (University of Milan, Milan, Italy). The study adhered to the tenets of the Declaration of Helsinki and was approved from the local institutional review board. Informed consent was obtained from all enrolled subjects after a thorough explanation of the study protocol.

Participants were recruited during the aforementioned period among the hospital staff (eg, nurses, doctors) and subjects attending the eye clinic for general ophthalmic examination. Consecutive eyes of participants fulfilling the inclusion criteria were included and examined under both normal (photopic) and pharmacologically induced mydriatic conditions. The inclusion criteria were as follows: (1) no history of uveitis or any ocular or systemic disease known to alter blood ocular barriers (eg, diabetes, glaucoma), (2) absence of corneal opacities that could impede adequate imaging of the AC, (3) no surgical procedures within the 6 months preceding the study, and (4) the ability to understand and willingness to sign the informed consent form. Both eyes of a participant were included if they met the inclusion criteria.

Procedures and Imaging Acquisition

All participants underwent a comprehensive ocular examination, which included collecting medical history, assessing best-corrected visual acuity, measuring intraocular pressure, and performing slit lamp biomicroscopy of the anterior segment to exclude the presence of clinically detectable inflammatory cells and pigment in the AC before pupil dilation. Relevant clinical data collected were sex, age, iris color [15] (blue, green, brown), and lens status (phakic, pseudophakic).

Each eye was initially imaged using the Anterion swept-source AS-OCT (Heidelberg Engineering GmbH) under photopic conditions (>10 cd/mm^2) before pupil dilation. The acquisition procedure involved selecting the "imaging" modality, enlarging the cross-sectional scan to 16.5 mm with a resolution of 768 a-scan or b-scan, and setting an averaging factor of 1 to obtain a single acquisition for each scan. For each acquisition, the participant was instructed to keep their eye open and steadily focus on the instrument's internal target during the entire acquisition time. A horizontal OCT b-scan of the AC, centered on the corneal apex and extending to the anterior capsule of the lens, was acquired and analyzed in this study. Each eye was randomly assigned to 1 of the 2 groups: pupil dilation (1) with 1 drop of phenylephrine 2.5% + tropicamide 0.5% (PH + T) or (2) with 1 drop of tropicamide 0.5% only (T). All images were reacquired 10 minutes after the administration of the mydriatic drops using the same scanning protocol.

A single eye from 1 patient was dilated on 2 separate occasions: once with T only and again, 7 days later, with a combination of PH + T. This eye was thus imaged under 3 conditions: (1) natural miosis, (2) dilation with T, and (3) dilation with PH + T. This eye was excluded from the statistical analysis and used to visually demonstrate the differential effects of the 2 mydriatic agents on the same eye.

Imaging Analysis

The single-line AS-OCT scans were exported as .pdf files from the Heidelberg Eye Explorer 2 (version 2.5.7) review software and converted into .tiff format for evaluation using FIJI, an open-source software based on ImageJ (Version 2.0.0-rc-69/1.52p, https://imagej.nih.gov/ij/).[16] In brief, after setting the scale (1877 pixels per 16.5 mm, µm/pixel ratio of 8.79), a rectangular horizontally-oriented region of interest (ROI) 1 was manually selected at the center of the scan, with the superior edge aligned with the corneal apex and the inferior edge resting on the anterior surface of the lens. The selected rectangle was then converted into an 8-bit image (Fig 1B), and the area of the AC was manually outlined by marking a horizontal line at the level of the iris as the inferior boundary (ROI 2; Fig 1C). We measured the AC area (ACA) (ROI 2) after excluding the reflection artifact seen as a hyperreflective curved band generated by corneal projection, as described by Naoya Yoshihara et al (ROI 3; Fig 1D).

Region of interest 3 was then processed for cell counting as follows. A binarization threshold was manually adjusted for each image to ensure the inclusion of the maximum number of HRDs (Fig 1C). The same manual threshold was applied to analyze the scan of the same eye after the administration of mydriatic drops. The identified particles were counted using the FIJI function called "Analyze Particles," which selects particles based on their size and circularity. Only particles >1 pixel^2 and with a circularity >0.5 were included in the analysis to exclude single-pixel random noise. Given our OCT imaging settings (~8.79 µm/pixel laterally), this cutoff translates to excluding particles

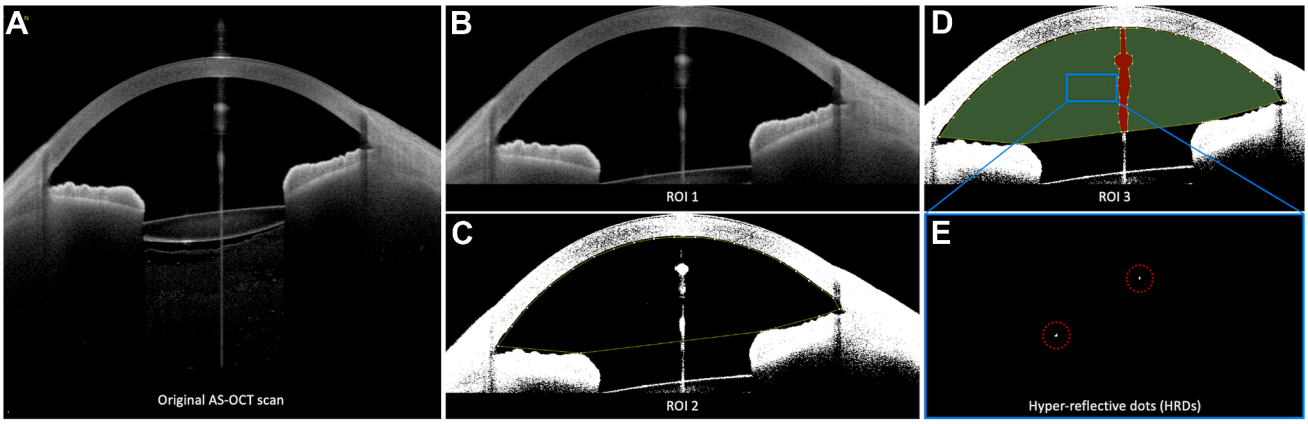

Figure 1. Postprocessing analysis for HRD detection on single-line AS-OCT scans. **A,** The original AS-OCT scan was converted from PDF to TIFF format for analysis using FIJI (National Institutes of Health). **B,** A rectangular ROI 1 was created with the corneal apex as the upper boundary and the anterior surface of the lens as the lower boundary. **C,** A second region (ROI 2) was defined after binarization with manual thresholding, outlining the area between the corneal endothelial surface and a horizontal plane at the level of the pupil margin (yellow contour). **D,** The ACA (green) was then calculated by subtracting the hyperreflective projection artifact generated by the cornea (red) (ROI 3) from ROI 2. **E,** Magnified view of a part of the anterior chamber (light blue square in **D**) showing 2 HRDs (red dotted circles). Hyperreflective dots >1 pixel^2 and with a circularity >0.5 were automatically detected in ACA and counted using the "Analyze Particles" tool. ACA = anterior chamber area; AS-OCT = anterior segment OCT; HRD = hyperreflective dot; ROI = region of interest

with an area smaller than ~77 μm^2, equivalent to a circular particle radius of ~5 μm. This approach ensures that analyzed HRDs represent biologically relevant particles (including cellular and noncellular elements)[12] rather than imaging noise or single-pixel artifacts (Fig 1E). Examples of raw and binarized images for further clearance are reported in Supplement Figure 1 (available at https://www.ophthalmologyscience.org). The HRD density was measured by dividing the total HRD number by the ACA and expressed as HRDs per 10 000 pixels^2 (corresponding to a 100 x 100 pixel area). Additionally, to account for the dynamic location of the iris within the AC and lens diaphragm, we manually measured the apex-to-pupil distance (APD), defined as the distance between the endothelial side of the corneal apex and a horizontal plane passing through the pupillary border.[17] This analysis was performed on all images acquired before and after pupil dilation. Binarization to include the maximum number of HRDs was performed by 2 independent ophthalmologists (P.M. and F.R.). Prior to this analysis, a third senior grader (A.I.) manually delineated the ACA (ROI 2) once for each image. Any discrepancies >20% between the binarization results of 2 graders were resolved by the same third grader. A subset of eyes (32 eyes from 24 participants) underwent multiple single-line OCT scans under different pupil conditions (miosis, T only, or PH + T). These repeated scans were utilized to assess the repeatability of the HRD measurements.

Statistical Analysis

Descriptive data were summarized using the mean (standard deviation [SD]), median (interquartile range), and number (percentages, %) where appropriate.

Demographic and clinical characteristics were compared between groups using *t* tests, chi-square tests, and Fisher tests as appropriate. Within-group changes from miosis to pharmacological dilation were assessed using paired Wilcoxon tests in both the T and PH + T groups.

A generalized Poisson mixed-effects model was used to estimate the expected density of HRDs in the AC. The main predictor in this model was the interaction between the mydriatic group (miosis, T, PH + T) and lens status (phakic, pseudophakic). The model was adjusted for age, sex, iris color, and variation in APD after dilation (APDD) as fixed effects, with outcomes nested within patients as random effects. Because the independent variable of this model was the absolute count of cells measured for each image, the area of the OCT image from which the count was obtained was included as an offset to derive the density of HRDs per 100 square pixels. To evaluate the proportion of eyes that after pupil dilation exceeded predilation values of cell density, we set a 3 SD threshold of the mean of miotic eyes' density. This threshold corresponds to 99.7% of the distribution of values in the myosis group. The 95% binomial confidence intervals (CIs) of the proportions were calculated with the Pearson−Klopper method. Results are presented as incidence rate ratios (IRRs) with 95% CIs and corresponding *P*-values. The repeatability of measurements (intergrader and intragrader agreement) was quantified using intraclass correlation coefficients with 95% CIs and visually represented with Bland−Altman plots, including 95% limits of agreement.

A *P* value of 0.05 was considered statistically significant. All analyses were conducted using R software version 4.2.2 (R Foundation for Statistical Computing, 2022).

Results

A total of 100 healthy eyes (59 participants) were included and randomly assigned to 2 groups: 52 eyes from 37 participants were dilated with both PH + T and 48 eyes from 32 participants were dilated with T only. The mean (SD) age of our cohort was 73 (11.7) years (range: 37−93), with 33 participants being female (55.9%). There were no significant differences between the 2 groups in terms of mean age, sex distribution, iris color, lens status, and density of HRDs per 10 000 pixels^2 (all $P > 0.05$). Both groups also showed similar average values of APD, ACA, and APDD (all $P > 0.05$). Both intragrader and intergrader agreements were excellent, with intraclass correlation coefficient values

of 0.97 (95% CI, 0.94−0.98) and 0.99 (95% CI, 0.99−1.00), respectively (both $P < 0.001$) (Supplement Figure 2, available at https://www.ophthalmologyscience.org).

The demographics, clinical characteristics, and imaging data regarding APD, ACA, and APDD for the 2 groups are reported in Table 1.

HRDs Density Variations after Dilation

The mean (SD) density of HRDs per 10 000 pixels^2 significantly increased from 0.79 (1.44) in normal photopic conditions to 1.71 (3.30) after the dilatation in T group ($P = 0.03$, r effect size = −0.377, Fig 2) and from 0.52 (0.83) to 15.8 (25.8) in the PH + T group ($P < 0.001$, r effect size = −0.753, Fig 2).

Regarding eyes exceeding the prespecified threshold (3 SD), 4.8% (95% CI, 0.1%−23.8%) of pseudophakic eyes and 22.2% (95% CI, 8.6%−42.3%) of phakic eyes in the T-only group showed a number of HRDs in the AC exceeding the 3-SD threshold after dilation compared with predilation values. In the PH + T group, 5.6% (95% CI, 0.1%−27.3%) of pseudophakic eyes and 58.8% (95% CI, 40.7%−75.4%) of phakic eyes exceeded the 3-SD threshold after dilation (Fig 3).

Associations between Ocular Parameters and HRDs

The generalized Poisson mixed-effects model revealed that the use of mydriatic drug, the APDD, and the interaction between the mydriatic drug and lens status significantly affected the expected HRDs density, after controlling for various confounders. Complete results from the Poisson model are provided in Table 2.

Specifically, dilation with either T or PH + T significantly influenced the expected HRDs density (IRR [95% CI], 1.6 (1.04, 2.47), $P = 0.03$, and 3.85 (2.31, 6.44), $P < 0.001$, respectively). An increase in APDD was also associated with a higher expected HRDs density (IRR [95% CI], 1.2 [1.12, 1.29], $P < 0.001$). Additionally, the significant interaction between dilation with PH + T and lens status revealed an additive effect, indicating that the use of PH + T in phakic eyes is associated with a significant increase in expected HRDs density beyond the sum of the individual effects (IRR [95% CI], 5.22 [2.43, 11.2], $P < 0.001$). Expected HRDs density values according to dilation group and lens status are detailed in Figure 3. The associations between the analyzed factors and the expected HRDs density are graphically presented in Figure 4. Figure 5 illustrates the single case where a patient underwent initial dilation with T, followed by dilation with PH + T 7 days later.

Discussion

In this study, we found that pharmacological pupil dilation significantly increases the number of HRDs observed in the AC of healthy individuals using AS-OCT. Because these round particles in the AC are typically considered inflammatory cells, this imaging approach is being investigated as a potential method for objectively assessing the amount of IOI. However, our results suggest that pupil mydriasis and lens status may introduce potential biases that should be accounted for while performing such measurements.

In recent years, there has been growing interest in identifying objective and repeatable biomarkers to measure IOI. Various strategies have been explored, including the development of new imaging biomarkers, such as choroidal

Table 1. Demographic and Clinical Characteristics of Included Eyes

	T	PH + T	P
Eyes, n	48	52	
Age, years—mean (SD)	71.8 (11.7)	73.2 (12.2)	0.55
Females, n (%)	32 (66.7%)	32 (61.5%)	0.47
APD, pixel—mean (SD)	385.5 (68.1)	377.9 (44.6)	0.51
APDD, pixel—mean (SD)	18.9 (37.3)	23.5 (36.7)	0.53
ACA, pixel^2—mean (SD)	20.1 (4.1)	19.8 (3.6)	0.69
HRDs density before pharmacological dilation, number per 10 000 pixel^2—mean (SD)	0.79 (1.44)	0.52 (0.83)	0.24
HRDs density after pharmacological dilation, number per 10 000 pixel^2—mean (SD)	1.71 (3.30)	15.8 (25.8)	**0.01**
Lens status, n (%)			0.47
Phakic	27 (56.2%)	34 (65.4%)	
Pseudophakic	21 (43.8%)	18 (34.6%)	
Iris color, n (%)			0.06
Brown	41 (85.4%)	34 (65.4%)	
Green	1 (2.1%)	14 (26.9%)	
Light blue	6 (12.5%)	4 (7.7%)	

ACA = anterior chamber area; APD = apex-to-pupil distance; APDD = apex-to-pupil distance after dilation; n = number; PH + T = phenylephrine + tropicamide; HRD = hyperreflective dot; SD = standard deviation; T = tropicamide only.
Bold value represents statistical significance ($P < 0.05$).

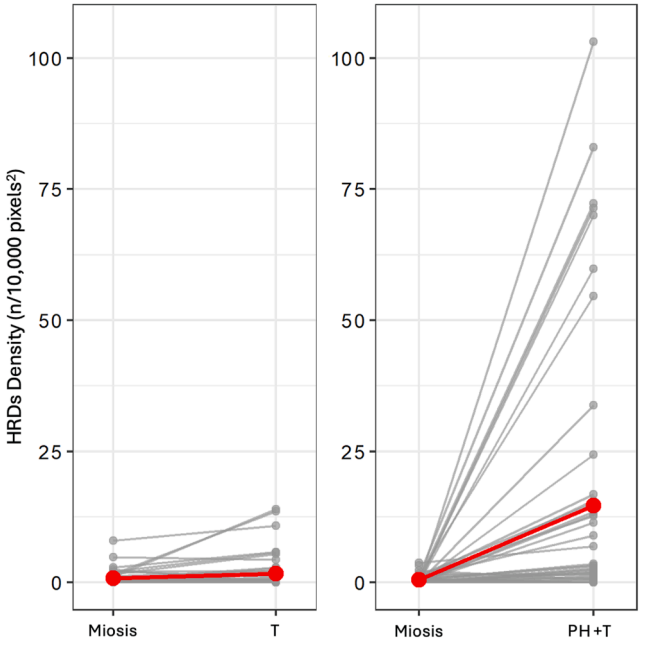

Figure 2. Changes in HRD density (number per 10 000 pixels^2) under different dilation conditions. The paired line plot in the left panel illustrates a modest increase in HRD density before (miosis) and after dilation with tropicamide 0.5% (T), whereas the right panel shows a marked increase in HRD density from miosis to dilation with PH 2.5% + T 0.5%. The red lines represent the overall mean trend across subjects, and the gray lines depict the trajectories for individual subjects. HRD = hyperreflective dot; PH + T = phenylephrine + tropicamide.

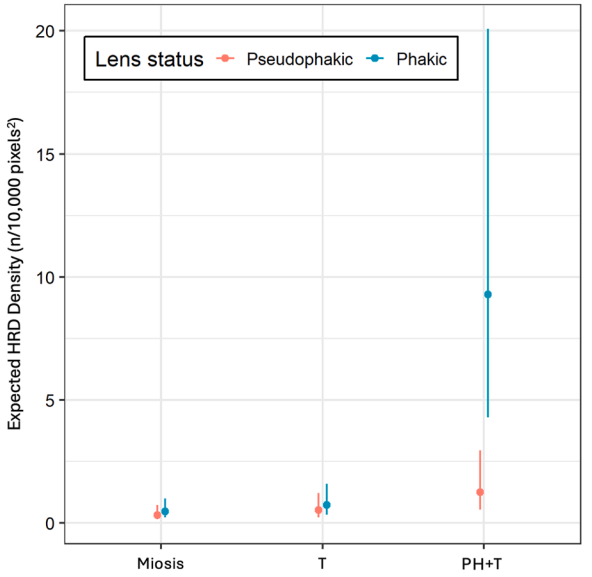

Figure 3. Expected HRD density (number per 10 000 pixels^2) across 3 dilation conditions, stratified by lens status. The central dots represent the mean estimated HRD density, with error bars indicating the 95% confidence intervals for each condition (miosis, dilation with tropicamide 0.5%, and dilation with PH 2.5% + T 0.5%). Red represents pseudophakic eyes, and blue represents phakic eyes. A pronounced increase in HRD density is observed after dilation with PH + T, particularly in phakic eyes. HRD = hyperreflective dot; PH + T = phenylephrine + tropicamide.

thickness and choroidal vascularity index, as well as the imaging-based quantification of established inflammatory markers, like inflammatory cells in the AC and vitreous cavity. Specifically, several studies have investigated the quantification of cells in the AC using AS-OCT images.[8–11]

Table 2. Clinical and Demographic Features Associated with an Increased Density of Hyperreflective Dots in Healthy Subjects

	Incidence Rate Ratios (95% CI)	*P*
Dilation		
Myosis (ref.)	1	
T	1.6 (1.04, 2.47)	0.03
PH + T	3.85 (2.31, 6.44)	<0.001
Lens status		
Pseudophakic (ref.)	1	
Phakic	1.43 (0.56, 3.61)	0.46
Dilation and lens status interaction		
T x Phakic	0.98 (0.52, 1.82)	0.94
PH + T x Phakic	5.22 (2.43, 11.2)	<0.001
Gender		
Female (ref.)	1	
Male	0.63 (0.27, 1.46)	0.28
Age (per 10 yrs)	1.09 (0.77, 1.53)	0.64
APDD	1.2 (1.12, 1.29)	<0.001
Iris color		
Brown (ref.)	1	
Green	1.9 (0.63, 5.73)	0.25
Light blue	0.43 (0.1, 1.88)	0.26

APDD = apex-to-pupil distance after dilation; CI = confidence interval; n = number; PH + T = phenylephrine + tropicamide; T = tropicamide only.

The use of AS-OCT to visualize inflammatory cells in the AC offers several advantages over the traditional slit lamp–based clinical examination. First, this approach is repeatable and less dependent on the operator. Second, cells on AS-OCT images can be counted on a continues scale, thus allowing for the detection of minimal count variations and potentially offering greater sensitivity than the 4-step Standardization of Uveitis Nomenclature scale.[18] Additionally, AS-OCT can easily bypass corneal opacifications, enabling cell measurement cells even when the visualization of AC content is clinically challenging.[19] However, this novel imaging approach has yet to be standardized, and the variables influencing these measurements must be identified and thoroughly investigated. Floating pigment fragments for instance share most OCT features with inflammatory cells, making it difficult to distinguish between the 2.[11] As such, any factor inducing pigment release into the AC could alter cells count and warrants further investigation.

In this study, 2 mydriatic eye drop formulations were used to obtain pupil dilation: PH 2.5% + T 0.5% and T 0.5% alone. Notably, PH + T demonstrated a significantly greater impact on the density of HRDs, compared with T alone. This result may be explained by the more pronounced dilation achieved with PH + T, which may cause the iris to shift further backward, potentially increasing contact with the lens or intraocular lens and leading to pigment release that mimics cells.[20] Notably, the increase in

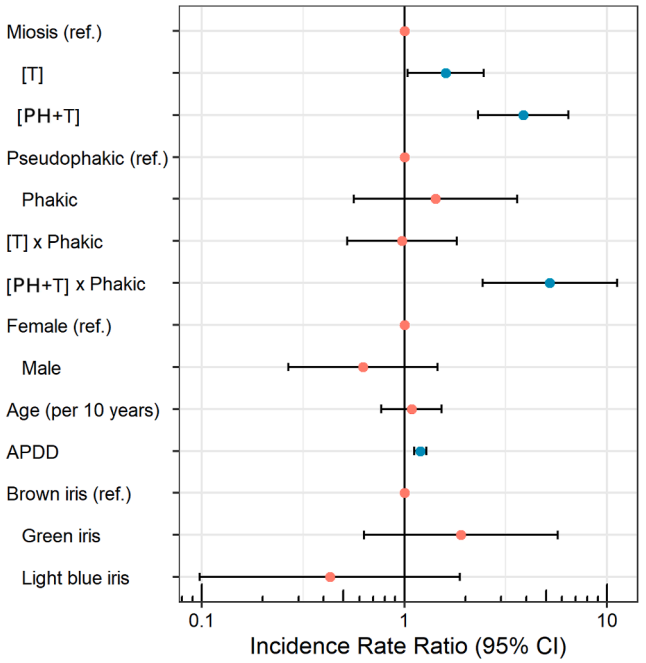

Figure 4. Generalized mixed-effects Poisson model assessing predictors of expected HRD density within the region of interest. The forest plot illustrates the IRRs and 95% CIs for variables predicting HRD density. Significant predictors include dilation with T 0.5%, PH 2.5% + T 0.5%, the interaction between PH + T and phakic lens status, and changes in the APDD. Blue dots indicate significant predictors, and the horizontal lines represent the 95% CI for each IRR. APDD = apex-to-pupil distance after dilation; CI = confidence interval; HRD = hyperreflective dot; IRR = incidence rate ratio; PH + T = phenylephrine + tropicamide; T = tropicamide only.

HRDs among patients dilated with PH + T was nearly 3 times higher than in those dilated with T alone (58.8% versus 22.2%).

Friction between the iris posterior surface and the anterior portion of the lens after pharmacological dilation should predominantly affect phakic eyes, whereas it would be virtually impossible in most pseudophakic eyes, except in cases with intraocular lens misplacement.[21] Our findings are in line with this explanation, as phakic eyes exhibited a more significant increase in HRDs compared with pseudophakic ones, regardless of the mydriatic agent used. Further evidence supporting this mechanism is the direct association between the increase in APDD and the number of HRDs visible on AS-OCT. In simpler terms, the greater the APDD, the more substantial the contact between the iris and the lens or intraocular lens was, ultimately resulting in increased pigment release into the AC after dilation.[12]

We do not have definitive evidence confirming that the HRDs observed in the AC after pharmacological dilation are pigment granules. However, current literature[12] and the mechanistic interpretation described above suggest that pigment particles likely account for the majority of these findings. Nevertheless, other elements, such as inflammatory cells or protein aggregates, may exhibit similar OCT characteristics and therefore contribute to the observed HRDs count, particularly in eyes with underlying inflammation. Further studies incorporating AC paracentesis and aqueous humor cytology are warranted to accurately determine the composition of these particles. Additionally, the application of artificial intelligence–based image analysis may offer a promising avenue to distinguish between the various types of hyperreflective elements that currently appear morphologically indistinguishable on OCT imaging.

Because pigment release appears to be the leading cause of the increase in HRDs in the AC after dilation, it would be reasonable to hypothesize that iris color might play a role, as darker irises contain more pigment. However, in our

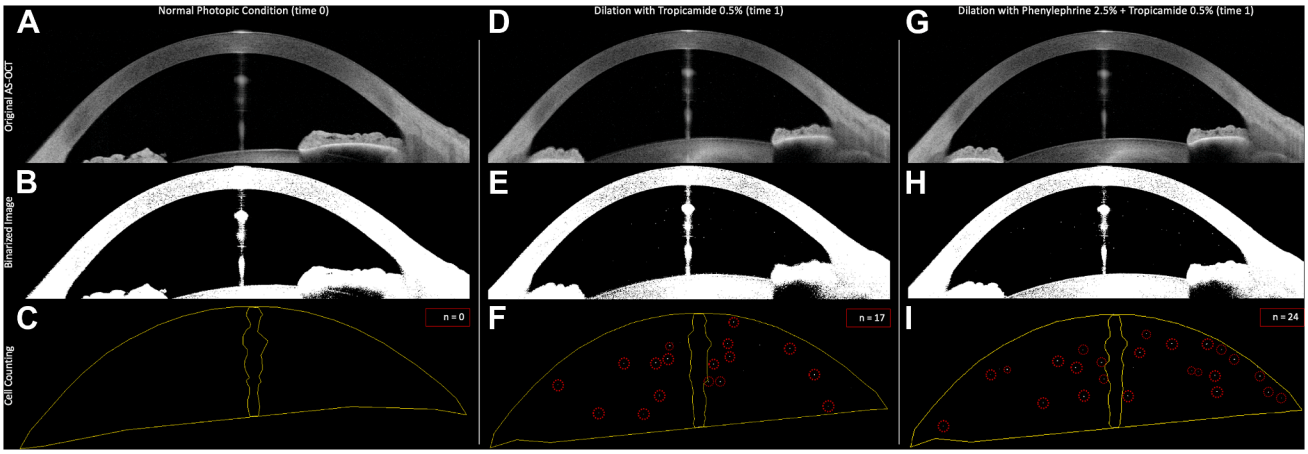

Figure 5. Variations in HRD counts in a single eye undergoing dilation with T 0.5% and, 7 days later, with PH 2.5% + T 0.5%. The first row displays the original AS-OCT scans, the second row shows the corresponding binarized images, and the third row highlights the cell counting maps. **A** to **C,** No visible HRDs in miosis under normal photopic conditions, while dilation with both T (**D–F**) and PH + T (**G–I**) resulted in an increased number of HRDs, with the highest count observed after PH + T administration. AS-OCT = anterior segment OCT; HRD = HRD = hyperreflective dot; PH + T = phenylephrine + tropicamide; T = tropicamide only.

study, iris color did not significantly influence the increase in HRDs density. This can be explained by considering the anatomy of the iris.[22] The posterior surface of the iris, which releases pigment due to friction with the lens, is composed of the iris pigment epithelium, a layer that is consistent across all eyes, regardless of iris color. In contrast, iris color is determined by the number of melanin granules contained in the iris stroma which is not in contract with the lens.[23]

We would like to acknowledge the limitations of our study. First, except for 1 patient, we dilated all the eyes with a single mydriatic eyedrop (either T or T + PH). Consequently, we do not have a direct comparison of the effect of these 2 agents on the same group; however, our 2 groups had similar demographic characteristics, thereby limiting statistical error. Second, the mean APD was manually calculated by tracing a line between the anterior surface of the iris and the posterior surface of the cornea, corresponding to the endothelium. We attempted to mitigate the impact of this nonautomatic calculation by considering the difference in APD before and after dilatation. Third, our study population included only nonuveitic eyes, with a mean subject age >70 years. While a similar effect of pharmacological dilation might be expected in younger individuals and eyes with uveitis, age-related lens thickening and inflammatory changes could increase friction between the lens and the iris. As a result, the magnitude of mydriasis-induced changes in HRD count and density may differ in uveitic populations and younger subjects.

Lastly, it should be noted that the ANTERION system does not have a follow-up function to reacquire the AS-OCT scans in the same location after dilation, which may have introduced variability in our measurements. While HRDs are constantly moving, thus making exact scan-rescan position less relevant for measurements repeatability, moving particles within the AC are not evenly distributed;[24] thus, their density may be slightly different depending on the scan location. In addition, cells and pigment granules freely move across the AC and moving elements have been reported to negatively affect measurements repeatability by other groups measuring cells in inflamed eyes.[25] In the current study we ran a repeatability analysis on a subset of subjects, and we had good intra- and intergrader agreement. This discrepancy in our favor compared with what reported in the past is likely related to the relative high number of eyes having zero particles in our population, thus inducing a quite relevant floor effect.

In conclusion, while the objective quantification of IOI using AS-OCT shows promise, several potential limitations must be carefully considered. Our study demonstrates that HRDs significantly increase after pharmacological dilation in healthy eyes, with PH + T exhibiting a stronger effect than T alone. Because these HRDs, likely corresponding to pigment fragments, are difficult to distinguish from inflammatory cells in the AC, they could introduce an important bias when using AS-OCT for assessing IOI. For this reason, we recommend assessing AC cells using AS-OCT either before dilation or under consistent pupil conditions across all visits. Further studies are necessary to confirm these findings and to standardize OCT-based measurements of AC inflammation.

Footnotes and Disclosures

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HUMAN SUBJECTS: Human subjects were included in this study. The study adhered to the tenets of the Declaration of Helsinki and was approved from the local institutional review board. Informed consent was obtained from all enrolled subjects after a thorough explanation of the study protocol.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Manni, Romano, Invernizzi

Data collection: Manni, Romano, Zaffalon, Barbieri, Zicarelli, Oldani, Chisari

Analysis and interpretation: Manni, Romano, Airaldi, Zicarelli, Cimino, Invernizzi

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Abbreviations and Acronyms:
AC = anterior chamber; **ACA** = anterior chamber area; **APD** = apex-to-pupil distance; **APDD** = apex-to-pupil distance after dilation; **AS-OCT** = anterior segment OCT; **CI** = confidence interval; **HRD** = hyperreflective dot; **IOI** = intraocular inflammation;

IRR = incidence rate ratio; **PH** = phenylephrine; **ROI** = region of interest; **SD** = standard deviation; **T** = tropicamide.

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