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Biomarkers for eyes with high injection demand in anti-angiogenic Therapy of nAMD

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Research Article

Keywords: Anti-angiogenic therapy, neovascular AMD, treatment frequency, macular neovascularization area (MNV)

Posted Date: November 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11259165/v1

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Additional Declarations: Competing interest reported. All AMD-Netz co-workers received funding for this study from Roche, Germany. Daniel Pauleikhoff also received consulting fees from Bayer, Novartis, Heidelberg engineering and Apellis.

Abstract

Purpose: Anti-VEGF therapy for neovascular age-related macular degeneration (nAMD) is a long-term treatment with individually varying injection requirements. Patients with high treatment demand could benefit from new therapeutic approaches involving continuous drug delivery. Suitable predictive biomarkerscould help identify them early.

Methods: In a cohort of 744 eyes (mean age 78.4 years, range 43-96; mean follow-up 3.3 years, range 1–10), injection demand was categorized into four groups: ≤4.5 injections/year (n=59), >4.5–≤6.5 (n=203), >6.5–≤8.5 (n=309), and >8.5 injections/year (n=173). Annual optical coherence tomography (OCT) examinations were available for all eyes, with a total of 18,743 OCT scans analyzed. Using recently published algorithms, macular neovascularization (MNV) area and growth as well as central retinal thickness (CRT) were quantified. ANOVA and linear models were applied to assess the relationship between injection frequency in the first year, long-term treatment demand, and morphological parameters. MNV growth was analyzed using grouped growth curve models.

Results: Injection frequency during the first year of treatment showed a significant association with long-term annual injection demand ($p<0.001$). Baseline MNV size and CRT were higher in eyes with higher injection frequency. In addition, MNV growth after one year as well as during follow-up was slower while a lower reduction of CRT was observed in eyes with higher injection frequency.

Conclusion: Baseline MNV size and higher CRT and especially injection frequency in the first year may be key biomarkers for high long-term treatment demand. They may facilitate early identification of patients who could benefit from continuous drug delivery therapies.

Key message

What is known: Anti-VEGF therapy for neovascular age-related macular degeneration (nAMD) is a long-term treatment with highly variable injection demand; early identification of eyes with persistently high demand is clinically relevant, e.g. for selecting patients for continuous drug delivery.

What is new: In 744 eyes, a higher baseline central retinal thickness (CRT) and a higher number of injections in the first treatment year were significantly associated with higher long-term injection demand, whereas baseline macular neovascularization (MNV) area showed only a non-significant trend.

What is new: Eyes with higher injection demand showed less MNV growth over up to six years, suggesting structural effects of more intensive anti-VEGF treatment beyond fluid resolution.

Introduction

Neovascular age-related macular degeneration (nAMD) is a leading cause of irreversible central vision loss in the elderly. The disease is characterized by the development of macular neovascularization (MNV), resulting in vascular leakage with intraretinal and/or subretinal fluid accumulation and

subsequent photoreceptor damage if left untreated[1]. Intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapy has become the standard treatment and effectively preserves vision in most patients[2].

Several anti-VEGF agents, including ranibizumab, aflibercept, bevacizumab (off-label), brolucizumab, and faricimab, have demonstrated comparable efficacy in controlling exudative disease activity[2–6]. Likewise, different treatment strategies, including pro re nata (PRN) and treat-and-extend (T&E), achieve similar long-term visual outcomes when applied consistently[3–6].

However, despite effective control of exudation, treatment usually needs to be continued for many years, with considerable interindividual variability in injection frequency.

Patients with persistently high injection demand represent a particular clinical challenge, because they experience greater treatment burden and account for a disproportionate share of healthcare resources. The emergence of longer-acting anti-VEGF agents, sustained drug delivery systems, and gene therapy further emphasizes the need to identify patients who are likely to require intensive long-term treatment. Reliable biomarkers predicting treatment demand would facilitate individualized treatment planning and patient selection for these emerging therapeutic approaches.

Although several clinical and OCT biomarkers have been associated with disease activity, robust predictors of long-term injection demand remain poorly defined. In particular, quantitative features of the underlying MNV have received limited attention, despite MNV being the biological substrate that drives exudation and necessitates repeated VEGF suppression.

Therefore, the present study investigated clinical and morphological biomarkers associated with long-term anti-VEGF treatment intensity in one of the largest multi-center real-world cohorts reported to date (744 eyes with validated long-term follow-up). Emphasis was placed on AI-assisted three-dimensional OCT quantification of MNV morphology, together with conventional retinal imaging biomarkers and first-year treatment requirements, to identify predictors of persistently high long-term injection demand.

Materials and Methods

Study Design and patient cohort

This retrospective, multicenter, observational cohort study was designed to identify associations over time between imaging biomarkers and long-term anti-VEGF treatment demand in patients with neovascular age-related macular degeneration (nAMD).

The study was hypothesis-generating and based on consecutive real-world data collected from ophthalmology practices in North Rhine-Westphalia, Germany between 2009 and 2023[7]. The inclusion criteria required the presence of exudative nAMD at baseline fulfilling the German requirements for anti-angiogenic treatment in nAMD of the German Professional Association of Ophthalmologists (BVA), the

German Ophthalmological Society (DOG) and the German Retina Society (RG)[2] demonstrating a minimum follow-up of one year, availability of high-quality macular OCT volume scans (≥ 6 × 6 mm) at baseline and at year 1, regular visits until the final visit and visual acuity at baseline and final visit, and complete documentation of anti-VEGF injections during follow-up. Because of the retrospective study design, patients with treatment interruptions or discontinuation - particularly those for organizational reasons (e.g., incomplete adherence due to travel difficulties or patient preference to continue treatment elsewhere), as well as those due to medical complications such as major hemorrhages - were excluded. Following quality control, 744 eyes were available for statistical analysis.

Treatment decisions were guided by OCT-based retreatment criteria following the IVAN PRN protocol[9]. According to this protocol, patients received three initial loading injections, followed by monthly monitoring visits. If new disease activity was detected on OCT during follow-up, a further cycle of three-monthly injections was administered. Retreatment decisions were made by multiple clinicians. Treatment demand was defined as the annualized number of intravitreal anti-VEGF injections during the complete observation period. For selected analyses, a different definition focussing on first year of treatment was used, defining treatment demand as the number of intravitreal anti-VEGF injections during the first year of treatment. Based on a previously characterized pilot cohort with long-term follow-up[8, 10], eyes were categorized into four predefined treatment-demand groups:

- ≤ 4.5 injections/year
- > 4.5 – ≤6.5 injections/year
- > 6.5 – ≤8.5 injections/year
- > 8.5 injections/year

These categories represent clinically relevant levels of long-term treatment intensity observed under routine clinical conditions.

Patient data were anonymized and included up to the end of year 2023. All patients provided written informed consent for antiangiogenic therapy. Due to the retrospective nature of the study and anonymized data evaluation, the requirement for informed consent for study participation was waived. The study was approved by the local Institutional Ethics Committee (Ethics Committee AEKWL 2017-033-f-S) and adhered to the principles of the Declaration of Helsinki. Anonymized data were accessed for research purposes beginning in 2024.

OCT Acquisition and Artificial Intelligence-Based Analysis of imaging biomarkers

Macular OCT volume scans were acquired using Spectralis spectral-domain OCT (Heidelberg Engineering, Heidelberg, Germany) and exported from the Heidelberg Eye Explorer (HEYEX) database. Standard scan protocols consisted of 6 × 6 mm volume scans containing up to 97 B-scans, a total of 18,743 OCT scans were analyzed.

Automated image analysis was performed using deep-learning algorithms developed in collaboration with the Institute for Artificial Intelligence in Medicine (IKIM), University Hospital Essen[11].

The algorithms automatically segmented retinal structures and pathological lesions, including hyperreflective material (HRM), retinal fluid compartments, retinal pigment epithelium, Bruch's membrane and choroid. Because HRM represents the fibrovascular component of macular neovascularization (MNV), HRM area was used as quantitative surrogate marker of MNV size. The agreement between AI-derived HRM measurements and manually delineated MNV area has previously been validated and demonstrated high concordance[12]. Therefore, the term “MNV” will be used throughout the statistical analysis and results sections. The automated segmentation framework allows reproducible quantification of pathological OCT features and provides objective biomarkers for the assessment of disease activity and treatment response.

To analyze a possible association between treatment intensity and morphological characteristics, HRM area (mm²) and central retinal thickness (CRT; µm) were used as imaging biomarkers. These were extracted at baseline and all available follow-up visits. Longitudinal analyses therefore included changes in HRM area and CRT during the first treatment year and throughout the complete follow-up period. To investigate a possible association the four predefined treatment-demand groups were compared with respect to baseline HRM area and CRT as well as with respect to change in HRM area between baseline/3 months and first year and with respect to change from baseline in CRT to 3 months and last available CRT value. Development of change from baseline in HRM area up to 6 years from baseline was investigated. Correlation between first year treatment demand and treatment demand from year two onwards was examined for a subpopulation of eyes.

Statistical Analysis

For statistical analyses, R (Version 4.5.1; R Core Team, 2025) was used. Continuous variables are presented as mean ± standard deviation or median (interquartile range), as appropriate. Categorical variables are summarized as frequencies and percentages.

Differences between treatment-demand groups in baseline biomarkers as well as changes from baseline within the first year of treatment were analyzed using analysis of variance (ANOVA) and analysis of covariance (ANCOVA) for change from 3 months to 1 year, controlling for baseline biomarker. Linear regression models and Kruskal–Wallis tests were used as a sensitivity analysis. Grouped growth-curve models using all available OCTs were used to examine the difference in course of mean MNV area over up to 6 years from baseline. 95-% pointwise and simultaneous confidence intervals comparing groups at time points 1, 2, 3, 4, 5 and 6 years were derived from this model. Changes from baseline in CRT to 3 months and last available values were examined using ANOVAs. Correlation between first year treatment demand and treatment demand from year two onwards was examined for a subpopulation of eyes having a follow-up greater than two years, using ANOVA and linear regression models.

Because it is generally not possible for the AI algorithm to distinguish between hyperreflective fluid and MNV at baseline, it yields higher MNV area values. After 3 months, this effect becomes significantly smaller due to resorption of MNV caused by exudation. For this reason, the baseline overestimation was statistically adjusted as follows: The difference between the median MNV areas at the 3-month time point and at baseline was determined in a separate cohort (N = 289) and subtracted from the baseline MNV area value for each eye. To avoid negative areas, these values were set to zero.

Because this study was exploratory and hypothesis-generating, all statistical tests were considered exploratory. Consequently, reported p-values should be interpreted as nominal without adjustment for multiple testing.

Results

1. **Study Population and Baseline Characteristics**

After application of inclusion criteria and quality control, 744 eyes were available for analysis. Analysis involving biomarkers at 3 months used 705 eyes, i.e. for 39 eyes no OCT was available at time point. Mean overall injection frequency was 7.13 ± 1.75 injections/year (median 7.22, range 1.1–12.4). When stratified by injection frequency during the entire follow-up, 59 eyes (7.9%) received ≤ 4.5 injections/year, 203 (27.3%) received > 4.5–6.5, 309 (41.5%) received > 6.5–8.5, and 173 (23.3%) received > 8.5 injections/year. When cohorts were defined based on the first treatment year the distribution was: ≤4.5 injections (n = 60, 8.1%), > 4.5–6.5 (n = 142, 19.1%), > 6.5–8.5 (n = 166, 22.3%), and > 8.5 (n = 376, 50.5%) injections in year 1.

Mean patient age was 78.4 ± 7.7 years (range 43–96), with 66.7% of patients were female. A trend towards younger age with higher injection frequency was observed for the cohort definition based on the entire follow-up (≤ 4.5: 80.7 ± 7.3 vs. >8.5: 76.1 ± 7.5 years), as well as for the cohort definition based on the first year (≤ 4.5: 81.6 ± 6.6 vs. >8.5: 77.1 ± 7.9 years). The mean follow-up was 3.29 ± 1.85 years, comparable across cohorts (Table 1).

Table 1

Baseline characteristics of the study cohort (n = 744) stratified by anti-VEGF injection-frequency group, calculated over the entire follow-up period and during the first treatment year

Parameter	**≤ 4.5 inj/y n = 59**	**> 4.5 – ≤ 6.5 inj/y n = 203**	**> 6.5 – ≤ 8.5 inj/y n = 309**	**> 8.5 inj/y n = 173**	**Overall n = 744**
Age, mean ± SD (years)	80.7 ± 7.3	79.8 ± 7.2	78.3 ± 7.9	76.1 ± 7.5	**78.4 ± 7.7**
Female, n (%)	46 (77.9%)	141 (69.5%)	209 (67.6%)	100 (57.8%)	**496 (66.7%)**
Follow-up, mean ± SD (years)	3.30 ± 1.98	3.17 ± 1.90	3.37 ± 1.88	3.30 ± 1.69	**3.29 ± 1.85**
Baseline MNV area, mean ± SD (mm²)	7.59 ± 6.34	8.73 ± 6.82	8.41 ± 6.50	9.04 ± 7.14	**8.58 ± 6.73**
Baseline CRT, mean ± SD (µm)	0.683 ± 0.115	0.711 ± 0.113	0.724 ± 0.125	0.745 ± 0.136	**0.722 ± 0.125**
Parameter	**≤ 4.5 inj/1st y n = 60**	**> 4.5 – ≤ 6.5 inj/1st y n = 142**	**> 6.5 – ≤ 8.5 inj/1st y n = 166**	**> 8.5 inj/1st y n = 376**	**Overall n = 744**
Age, mean ± SD (years)	81.6 ± 6.6	79.5 ± 7.3	79.2 ± 7.4	77.1 ± 7.9	**78.4 ± 7.7**
Female, n (%)	44 (73.3%)	91 (64.1%)	116 (69.9%)	245 (65.2%)	**496 (66.7%)**
Follow-up, mean ± SD (years)	2.89 ± 1.74	3.17 ± 1.98	3.09 ± 1.57	3.49 ± 1.91	**3.29 ± 1.85**
Baseline MNV area, mean ± SD (mm²)	8.12 ± 6.72	8.45 ± 6.50	7.96 ± 6.50	8.97 ± 7.17	**8.58 ± 6.73**
Baseline CRT, mean ± SD (µm)	0.681 ± 0.112	0.702 ± 0.119	0.714 ± 0.126	0.740 ± 0.126	**0.722 ± 0.125**

CRT, central retinal thickness; MNV, macular neovascularization; inj/y, injections per year; inj/1st y, number of injections in first year.

1. **Analysis of the association between treatment-demand groups and morphologic biomarkers**

Baseline MNV area showed a trend towards larger lesions in eyes requiring higher long-term anti-VEGF treatment intensity. Although this association did not reach statistical significance, eyes in the groups with highest injection-frequency during the first year consistently exhibited larger baseline MNV measurements than eyes requiring fewer injections (p = 0.39). A similar pattern was found when calculating treatment demand over the entire follow-up (FU) (p = 0.49) (Fig. 1).

In contrast, CRT at baseline was significantly associated with future treatment demand. Eyes requiring more frequent anti-VEGF injections demonstrated significantly greater baseline CRT both in the one-year analysis (p < 0.001) and over the entire follow-up period (p = 0.004) (Table 2).

Table 2
Pairwise comparison of baseline central retinal thickness (CRT) at baseline between injection-frequency groups (ANOVA)

Injection demand calculated from entire Follow-up (overall p = 0.004)**			
Comparison	Difference[+]	95% CI	p-value
(> 8.5 inj/y) vs (≤ 4.5 inj/y)	0.062	[0.025, 0.098]	0.001**
(> 8.5 inj/y) vs (> 4.5 and ≤ 6.5 inj/y)	0.034	[0.009, 0.059]	0.009**
(> 8.5 inj/y) vs (> 6.5 and ≤ 8.5 inj/y)	0.020	[-0.003, 0.043]	0.087
(> 6.5 and ≤ 8.5 inj/y) vs (≤ 4.5 inj/y)	0.041	[0.007, 0.076]	0.019*
(> 6.5 and ≤ 8.5 inj/y) vs (> 4.5 and ≤ 6.5 inj/y)	0.014	[-0.008, 0.036]	0.2
(> 4.5 and ≤ 6.5 inj/y) vs (≤ 4.5 inj/y)	0.028	[-0.008, 0.064]	0.13
Injection demand calculated from 1st year (overall p < 0.001***)			
Comparison	Difference[+]	95% CI	p-value
(> 8.5 inj/1st y) vs (≤ 4.5 inj/1st y)	0.059	[0.025, 0.093]	< 0.001***
(> 8.5 inj/1st y) vs (> 4.5 and ≤ 6.5 inj/1st y)	0.038	[0.014, 0.062]	0.002**
(> 8.5 inj/1st y) vs (> 6.5 and ≤ 8.5 inj/1st y)	0.027	[0.004, 0.049]	0.022*
(> 6.5 and ≤ 8.5 inj/1st y) vs (≤ 4.5 inj/1st y)	0.032	[-0.004, 0.069]	0.083
(> 6.5 and ≤ 8.5 inj/1st y) vs (> 4.5 and ≤ 6.5 inj/1st y)	0.012	[-0.016, 0.039]	0.4
(> 4.5 and ≤ 6.5 inj/1st y) vs (≤ 4.5 inj/1st y)	0.021	[-0.017, 0.058]	0.3
* = p < 0.05, ** = p < 0.01, *** = p < 0.001; N = 744			

During the first year of treatment, growth of MNV area tended to be lower in eyes with higher injection frequencies. When MNV growth from baseline to one year was analyzed, an overall trend towards

reduced lesion growth with increasing treatment intensity was observed (p = 0.10), with several pairwise group comparisons reaching nominal statistical significance (Table 3). A similar pattern was observed for MNV growth between three months and one year, although this association was not statistically significant (p = 0.43). Sensitivity analysis via linear regression using raw number of injections within the first year as well as nonparametric Kruskal–Wallis tests showed consistency of these results. Over the complete follow-up period, eyes receiving more intensive anti-VEGF treatment demonstrated significantly less MNV growth than eyes requiring fewer injections (i.e., annual simultaneous confidence intervals for comparison of highest vs. lowest injection-demand excluded zero), confirming the long-term association between higher treatment intensity and reduced lesion expansion (Fig. 2).

Table 3
Pairwise comparison of change in macular neovascularization (MNV) area from baseline to year 1 between injection-frequency groups defined by first-year treatment demand (ANOVA)

Change from Baseline to 1 year (overall p = 0.1)			
Comparison	Difference[+]	95% CI	p-value
(> 8.5 inj/1st y) vs (≤ 4.5 inj/1st y)	-0.546	[-1.691, 0.598]	0.3
(> 8.5 inj/1st y) vs (> 4.5 and ≤ 6.5 inj/1st y)	-0.816	[-1.627, -0.005]	0.048*
(> 8.5 inj/1st y) vs (> 6.5 and ≤ 8.5 inj/1st y)	-0.785	[-1.552, -0.018]	0.045*
(> 6.5 and ≤ 8.5 inj/1st y) vs (≤ 4.5 inj/1st y)	0.238	[-1.002, 1.478]	0.7
(> 6.5 and ≤ 8.5 inj/1st y) vs (> 4.5 and ≤ 6.5 inj/1st y)	-0.031	[-0.972, 0.909]	> 0.9
(> 4.5 and ≤ 6.5 inj/1st y) vs (≤ 4.5 inj/1st y)	0.270	[-0.998, 1.537]	0.7
* = p < 0.05, ** = p < 0.01, *** = p < 0.001; N = 744			

Reduction in CRT during the first three months showed no significant differences between treatment-demand groups (p = 0.57), although a tendency towards greater retinal thinning with increasing injection frequency was observed, except for the highest treatment-demand group.

In the analysis based on first-year injection demand, however, CRT reduction from baseline to 3 months was significantly lower in eyes requiring more frequent anti-VEGF injections (p = 0.007; Table 4). In contrast, changes from baseline to the last available examination did not differ significantly between treatment-demand groups, neither in the one-year analysis (p = 0.74) nor over the complete follow-up period (p = 0.23). Residual diagnostics of all AN(C)OVA and linear regression models were unsuspicious with respect to heteroscedasticity and normality. Though deviations from normality were observed, analysis can well rely on normal approximation due to large sample size.

Table 4
Pairwise comparison of change in central retinal thickness (CRT) from baseline to month 3 between injection-frequency groups defined by first-year treatment demand (ANOVA)

Change from Baseline to month 3 (overall p = 0.007)**			
Comparison	Difference[+]	95% CI	p-value
(> 8.5 inj/1st y) vs (≤ 4.5 inj/1st y)	0.025	[-0.007, 0.058]	0.13
(> 8.5 inj/1st y) vs (> 4.5 and ≤ 6.5 inj/1st y)	0.038	[0.016, 0.060]	< 0.001***
(> 8.5 inj/1st y) vs (> 6.5 and ≤ 8.5 inj/1st y)	0.029	[0.009, 0.049]	0.005**
(> 6.5 and ≤ 8.5 inj/1st y) vs (≤ 4.5 inj/1st y)	-0.004	[-0.035, 0.027]	0.8
(> 6.5 and ≤ 8.5 inj/1st y) vs (> 4.5 and ≤ 6.5 inj/1st y)	0.009	[-0.011, 0.029]	0.4
(> 4.5 and ≤ 6.5 inj/1st y) vs (≤ 4.5 inj/1st y)	-0.013	[-0.045, 0.019]	0.4
* = p < 0.05, ** = p < 0.01, *** = p < 0.001; N = 744			

1. **Predictive value of first-Year treatment demand**

Analysis of correlation between injection frequency during the first treatment year with long-term treatment demand included 555 eyes. Results show a strong association with long-term treatment demand. Eyes requiring more injections during the initial year continued to require significantly higher injection frequencies throughout subsequent follow-up ($p < 0.001$). This relationship was highly significant across most pairwise comparisons, indicating that treatment intensity during the first year is a robust predictor of future anti-VEGF treatment requirements (Table 5).

Table 5
Association between injection demand during the first treatment year and injection demand from the second year onwards (linear regression and ANOVA)

Linear regression for injection demand from 2nd year onwards ~ injection demand 1st year (R^2 = 0.163)			
Predictor	Estimate	95% CI	p-value
Injection demand 1st year	0.355	[0.288, 0.422]	< 0.001***
ANOVA for injection demand from 2nd year onwards ~ injection demand 1st year (overall p < 0.001***)			
(1 to 3 inj/1st y) vs (4 to 6 inj/1st y)	-0.177	[-0.878, 0.524]	0.6
(1 to 3 inj/1st y) vs (7 to 9 inj/1st y)	-1.198	[-1.838, -0.558]	< 0.001***
(1 to 3 inj/1st y) vs (> 10 inj/1st y)	-2.602	[-3.276, -1.927]	< 0.001***
(4 to 6 inj/1st y) vs (7 to 9 inj/1st y)	-1.022	[-1.437, -0.606]	< 0.001***
(4 to 6 inj/1st y) vs (> 10 inj/1st y)	-2.425	[-2.893, -1.958]	< 0.001***
(7 to 9 inj/1st y) vs (> 10 inj/1st y)	-1.404	[-1.773, -1.034]	< 0.001***
* = p < 0.05, ** = p < 0.01, *** = p < 0.001; N = 555			

Discussion

The present study investigated imaging biomarkers associated with long-term anti-angiogenic treatment demand in a large real-world cohort of patients with neovascular age-related macular degeneration (nAMD). The principal findings were that a larger baseline HRM area, representing MNV, and a greater baseline CRT were associated with higher long-term injection requirements. Moreover, eyes requiring more intensive treatment demonstrated significantly less MNV growth and a lower reduction in CRT during both the first treatment year and the entire follow-up period. Finally, injection frequency during the first treatment year was a strong predictor of future treatment demand, indicating that the individual treatment requirement becomes apparent early during therapy and remains remarkably stable over time.

The baseline associations suggest that the morphological characteristics of the lesion at treatment initiation already reflect the biological activity of the disease. Larger MNV lesions and increased retinal thickness at baseline are likely to represent a higher degree of VEGF-driven exudative activity, resulting in a greater need for repeated VEGF suppression. Although baseline MNV showed only a statistical trend, the consistent increase across treatment-demand groups supports its potential value as an imaging biomarker. By contrast, baseline CRT demonstrated a robust association with future treatment intensity and therefore appears to be a more immediately applicable clinical predictor. This is consistent with recent findings that central subfield thickness assessed after the initial loading phase, rather than at baseline, predicts both injection frequency and recurrence timing in eyes treated with a treat-and-extend aflibercept regimen, further supporting the value of early post-loading morphology over baseline

measurements for guiding individualized treatment planning[13]. Nevertheless, neither biomarker alone is likely to provide sufficient predictive accuracy. Future prediction models will probably require the combination of several imaging biomarkers together with clinical and demographic variables. Machine-learning approaches applied to early OCT data acquired during treat-and-extend regimens have already demonstrated feasibility for this type of multivariable prediction[14]. Comparable machine-learning frameworks incorporating explainable feature-importance analysis (SHapley Additive exPlanations, SHAP) have shown similar potential for session-by-session prediction of anti-VEGF retreatment needs [15], and deep-learning models applied to paired early OCT scans have been used to forecast treat-and-extend intervals and visual outcomes[16]. Beyond structural OCT parameters, optical coherence tomography angiography (OCTA)-derived vascular architecture features of the MNV, including lesion area, total vascular length, and fractal dimension, have also been shown to predict long-term injection frequency and may represent a complementary, mechanistically distinct biomarker avenue for such multivariable models[17].

One of the most clinically relevant observations was the strong association between injection frequency during the first treatment year and subsequent long-term treatment demand. Eyes requiring more than 8.5 injections during the initial year continued to require intensive therapy throughout follow-up. This finding suggests that the individual exudative phenotype of nAMD is established early after treatment initiation and remains relatively constant despite prolonged anti-VEGF therapy as previously discussed by Gunnemann et al[8]. Such patients may therefore represent the subgroup most likely to benefit from sustained drug-delivery strategies, including long-acting delivery systems or future continuous drug-release approaches, as these treatments could substantially reduce treatment burden while maintaining disease control.

Interestingly, higher treatment intensity was consistently associated with significantly less HRM growth throughout follow-up. Because HRM has been validated as a surrogate marker of the fibrovascular component of MNV, this finding suggests that sustained VEGF suppression may not only control exudation but also influence the structural evolution of the neovascular lesion itself. In addition to its anti-permeability effect, intensive anti-VEGF therapy may modify the biological behaviour of MNV by limiting its further expansion and promoting stabilization of the neovascular complex. The results of previous studies by Pauleikhoff and colleagues [10] as well as by Cozzi et al[18] demonstrated MNV growth only under PRN, but not under T&E treatment strategy. This could also be interpreted as suggesting that recurrent 'spikes' in VEGF levels between injections, which are more common in a PRN treatment strategy, may drive MNV growth.

However, the smaller reduction in CRT observed in eyes requiring the highest injection frequency may be related to their larger baseline MNV size and greater baseline CRT. This finding may indicate that, in eyes with larger and more active MNV lesions, even intensive anti-VEGF treatment primarily stabilizes the overall disease state, rather than achieving a total reduction of associated exudative fluid. Whether more continuous drug exposure could further improve anatomical outcomes in these eyes remains to be investigated. Nevertheless, these results once again emphasize the importance of detecting treatment-

requiring MNV at an early stage, before extensive lesion growth and marked retinal thickening have occurred.

In addition, whether the stabilization of MNV growth represents a favourable vascular remodeling process or contributes to subsequent fibrotic transformation and ultimately macular atrophy (MA) remains uncertain. Previous longitudinal studies have observed the development of MA as a frequent and specific morphological feature during effective anti-angiogenic therapy in nAMD [3, 6, 19–21] which was independent of a reactive PRN or T&E treatment strategy [22]. It is therefore possible that more intensive VEGF suppression modifies the natural history of MNV by shifting the balance from active neovascular proliferation towards a more quiescent fibrovascular phenotype. Future AI-based longitudinal analyses integrating volumetric MNV measurements, fibrosis and macular atrophy measurements will be required to determine whether this biological transformation ultimately preserves retinal function or accelerates degenerative tissue remodeling.

The present study has several important strengths. To our knowledge, this represents one of the largest real-world imaging cohorts evaluating biomarkers of long-term anti-VEGF treatment demand. Automated deep-learning-based quantification of HRM and CRT enabled standardized and reproducible assessment of imaging biomarkers across a large number of OCT volume scans, minimizing observer-dependent variability and allowing analyses that would not have been feasible using manual measurements alone.

But several limitations should also be acknowledged. First, the retrospective design and the use of routinely collected clinical data make the analyses exploratory and hypothesis-generating. Consequently, all reported p-values should be interpreted as nominal. Secondly, HRM represents a validated surrogate rather than a direct measurement of MNV. Although previous validation demonstrated excellent agreement between AI-derived HRM and manually delineated MNV, baseline HRM measurements may be slightly overestimated because hyperreflective exudative components (subretinal hyperreflective fluid) cannot be completely distinguished from fibrovascular tissue[12]. To account for this, we introduced a statistical adjustment which included that negative values of MNV area after adjustment were set to zero. This alters baseline distributions within groups, but only very slightly, and the results with respect to group differences differ only minimally from results obtained without adjustment. Thirdly, longitudinal analyses based on mixed-effects growth-curve models are limited by progressive loss to follow-up. Although mixed-effects modelling appropriately handles missing observations under a missing-at-random assumption, informative dropout cannot be excluded in this long-term real-world cohort and may have influenced the estimated longitudinal trajectories. Finally, cohort allocation was based on treatment intensity observed during follow-up rather than on baseline characteristics alone, introducing the possibility of residual confounding and limiting causal interpretation of the observed associations.

In conclusion, this study identifies baseline HRM/MNV area and CRT as well as, most importantly, treatment intensity during the first year as promising biomarkers of future anti-VEGF treatment demand in neovascular AMD. Intensive treatment was consistently associated with reduced lesion growth and

more pronounced anatomical improvement, suggesting favourable structural effects beyond fluid resolution. These findings provide a foundation for future AI-based multivariable prediction models capable of identifying patients requiring sustained intensive therapy at an early stage. Whether early identification of these patients and implementation of continuous drug-delivery strategies can improve long-term visual outcomes or reduce the development of macular atrophy has also to be addressed in future studies.

Declarations

Funding: Roche Germany

Competing interests: The authors declare no competing interests.

Ethics approval: This retrospective study was approved by the local Institutional Ethics Committee (Ethics Committee AEKWL 2017-033-f-S) and adhered to the tenets of the Declaration of Helsinki.

Consent to participate: All patients provided written informed consent for antiangiogenic therapy. Due to the retrospective nature of the study and the use of anonymized data, the requirement for separate informed consent for study participation was waived by the Institutional Ethics Committee.

Consent for publication: Not applicable

Data availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions: All authors contributed to the study conception and design. [Jana Stasch-Bouws, Benja Vahldiek, Lukas Heine, Anna Vahldiek, Raphael Welsow, Frederic Jonske, Daniel Scholtyssek, Jens Kleesiek, Melissa Meyer zu Hörste] and [Daniel Pauleikhoff]: data curation and formal analysis. [Benja Vahldiek, Lukas Heine, Anna Vahldiek, Raphael Welsow, Frederic Jonske, Daniel Scholtyssek and Jens Kleesieck]: Development and validation of the AI segmentation algorithm. [Michael Swora]: statistical analysis. [Melissa Meyer zu Hörste] and [Daniel Pauleikhoff]: writing – original draft preparation. All authors read and approved the final manuscript.

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Figures

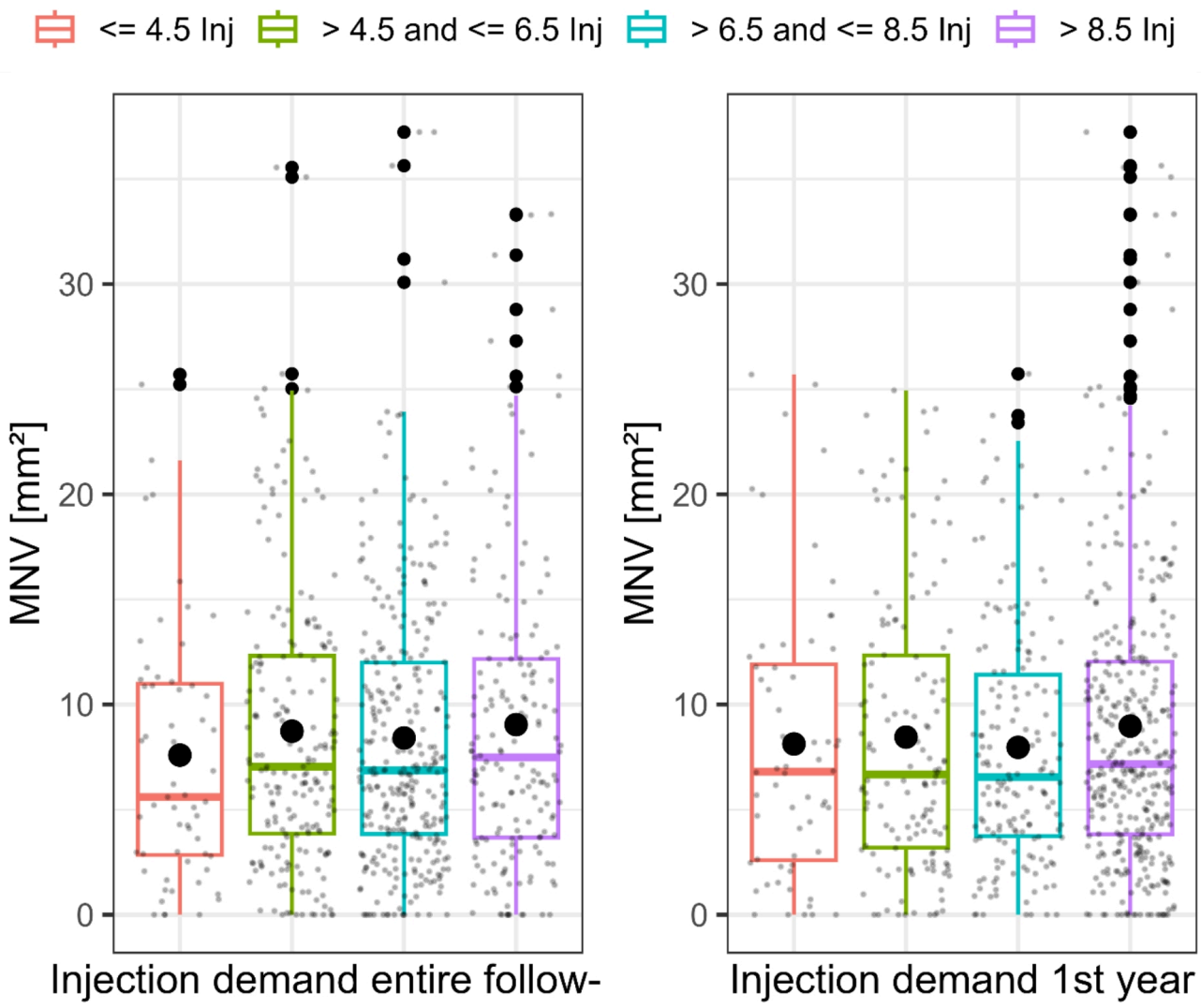


Figure 1

Baseline macular neovascularization (MNV) area by injection-frequency group, calculated over the entire follow-up period (left) and during the first treatment year (right). Boxes show median and interquartile range, large black dots the group mean, small grey dots individual eyes; inj, injections. Larger lesions were observed with increasing injection frequency, but the association did not reach statistical significance (entire follow-up: p = 0.49; first year: p = 0.39)

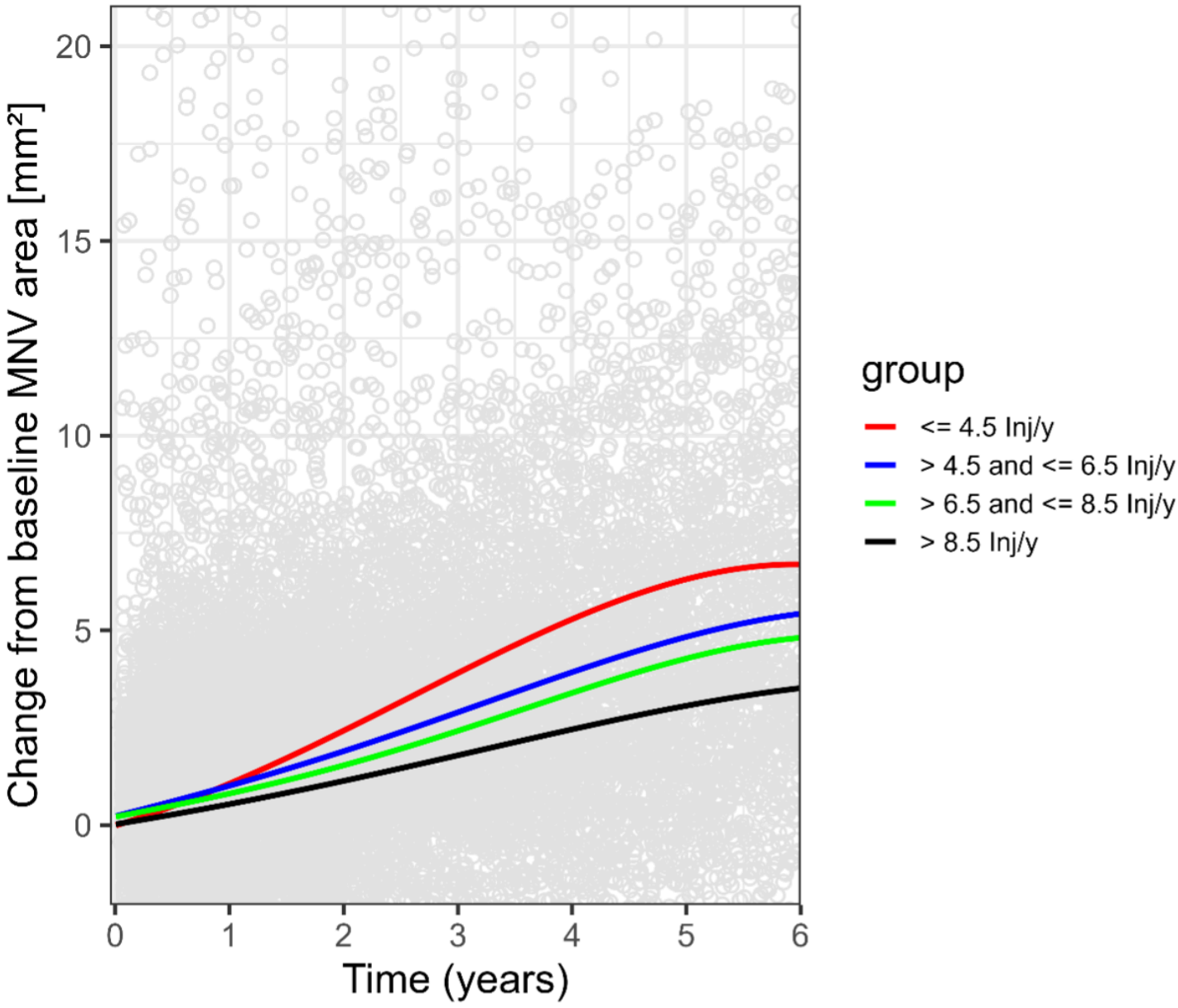


Figure 2

Growth of macular neovascularization (MNV) area (change from baseline, mm²) over the follow-up period by injection-frequency group. Lines show the fitted group means of the grouped growth curve model, grey circles individual measurements; inj/y, injections per year. Eyes with higher injection frequency showed significantly less MNV growth than eyes with lower injection frequency