• Clinical Research •

Comparison of two different treatment regimens' efficacy in neovascular age-related macular degeneration in Turkish population—based on real life data-Bosphorus RWE Study Group

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Abstract

● **AIM:** To compare two different anti-vascular endothelial growth factor (anti-VEGF) treatment regimens'-a priori *pro re nata* (PRN) and PRN regimen following the loading phase-anatomical and functional results in neovascular age-related macular degeneration (nAMD) patients.

● **METHODS:** Totally 544 nAMD patients followed and treated with aflibercept (*n*=135) and ranibizumab (*n*=409) at 9 different centers between 2013 and 2015 were enrolled into this retrospective multicenter study. Patients with initial best corrected visual acuity (BCVA) interval of 1.3-0.3 (logMAR) and a minimum follow-up of 12mo were included. Patients under two different regimens-a priori *pro re nata* (1+PRN) or 3 consecutive intravitreal injections followed by a PRN regimen (3+PRN)-were compared in BCVA at 3th, 6th and 12th months, and in central macular thickness (CMT) at 6th and 12th months. The total study group, intravitreal ranibizumab (IVR) and intravitreal aflibercept (IVA) groups were evaluated separately.

● **RESULTS:** The mean CMT decreased in the 1+PRN (*n*=101) regimen from 407 to 358 and 340 μm and in the 3+PRN (*n*=443) group from 398 to 318 and finally to 310 μm at months 6 and 12, respectively. Anatomically, the CMT reduction at 6th month (48.5 *vs* 76.4; *P*<0.05) was statistically significant in favor of 3+PRN group. BCVA changed in 1+PRN group from 0.77 to 0.78, 0.75 and 0.75; in 3+PRN group from 0.81 to 0.69, 0.72, and 0.76 at months 3, 6, and 12, respectively. Visual gain was statistically better in 3+PRN group at 3th month (-0.01 *vs* 0.12; *P*<0.001). In IVR group, CMT reduction was in greater in 3+PRN at 6th (44 *vs* 72) and 12th month (61 *vs* 84), but statistically insignificant. The 3+PRN group revealed statistically better visual results at 3th month (-0.02 *vs* 0.11, *P*<0.05). In IVA group, although statistically insignificant, CMT reduction (61 *vs* 89, 6th month; 85 *vs* 97, 12th month) and visual gain (0.02 *vs* 0.16; 0.02 *vs* 0.14; 0.05 *vs* 0.11) was found in favor of 3+PRN group at all visits.

● **CONCLUSION:** The loading dose of anti-VEGF treatments in nAMD leads to significantly better anatomical and functional results, regardless of the agent, specially in early follow-up interval.

● **KEYWORDS:** aflibercept; neovascular age-related macular degeneration; ranibizumab; loading dose; treatment regimen
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INTRODUCTION

Age-related macular degeneration (AMD) is the most common cause of vision loss and legal blindness in the population with age over 60y in the developed countries[1-2]. The AMD is divided into two major clinical subgroups according to the presence or absence of a choroidal neovascular membrane (CNVM); dry AMD subgroup starts with the developing of drusen in the posterior pole, progresses in severe cases to geographical atrophy at the fovea leading to severe visual impairment, the neovascular subgroup on the other hand is landmarked with the presence of a CNVM of various presentations. Although only 10%-20% of AMD patients are of neovascular type, it is responsible for severe vision loss or blindness in approximately 90% of AMD cases. Historically, photodynamic therapy was the first critical milestone to stop the progression of neovascular AMD (nAMD) at the beginning of this century[3]. Intravitreal pegaptanip sodium was first anti-vascular endothelial growth factor (anti-VEGF) drug introduced into the AMD treatment armamentorium[4]. Its promising results compared to the standard care were followed by off-label bevacizumab[5], on-label ranibizumab and aflibercept in search for better clinical outcomes for this devastating disease. Nowadays, anti-VEGF therapy is the most effective treatment option of nAMD.

Several pivotal clinical trials[6-8] suggested strict monthly treatment regimens for intravitreal anti-VEGF administration, accompanied with a close monitoring in nAMD patients[4,6], but in clinicians' daily praxis this strict protocol mostly failed due to several technical reasons. The economical and social burden of such a chronic therapy, the over-loading effect of endless treatment sessions and monitoring visits on patients and retinal physicians inhibited all participants from practicing monthly regimens. In the current study, we aimed to evaluate the treatment outcomes from nine tertiary retinal centers and to investigate the effect of initial regimen preferences based on our real life experience.

SUBJECTS AND METHODS

Ethical Approval Written informed consent was obtained from all patients before all invasive procedures in the follow-up and the study adhered to the tenets of the Declaration of Helsinki. Ethical board approval was obtained from Faculty of Medicine, Kocaeli University.

This is a multicenter, retrospective, observational, comparative real-life experience study, conducted in 9 tertiary centers in Istanbul and Kocaeli/Turkey. The records of treatment naive nAMD patients who were treated for the first time with an anti-VEGF—either ranibizumab or aflibercept-agent between January 2013 and December 2015 were reviewed by the investigators. Patients were divided into two major groups according to their treatment initiation regimen. The patients who were started with a priori *pro re nata* (PRN; as needed) treatment regimen were contributed into the 1+PRN group, on the other hand, patients who underwent a loading phase with three consecutive injections followed by a PRN regimen were enrolled into the 3+PRN group.

Patient Enrollment and Follow-up Our major inclusion criteria were being age of ≥50y, a diagnoses of nAMD, a minimum follow-up time of 12mo and having a baseline best corrected visual acuity (BCVA) within the range of 1.3-0.3 in logaritm of minimum angle resolution (logMAR). Patients who had co-existing retinal diseases other than nAMD (*e.g.*, diabetic retinopathy, retinal vein occlusion) or those diagnosed with polypoidal choroidal vasculopathy or retinal angiomatous proliferation or patients who were switched from one anti-VEGF drug to another during the study period were excluded from the study. Patients who were treated before the enrollment with any treatment modality and patients with irregular follow-up exceeding monthly/bimonthly visits were also excluded from the study group. According to our exclusion and inclusion criteria, 544 out of 783 nAMD patients [135 intravitreal aflibercept (IVA), 409 intravitreal ranibizumab (IVR)] were finally enrolled into the current study.

All eligible patients underwent comprehensive ophthalmological examination including BCVA measurement in Snellen ratios or the Early Treatment Diabetic Retinopathy Study (ETDRS) letters, slit-lamp biomicroscopy and fundus examination, and intraocular pressure measurement *via* Goldmann applanation tonometry at pretreatment, months 3, 6 and 12 visits. Fluorescein angiography (FA), and spectral domain optical coherence tomography (SD-OCT) imaging were performed before treatment initiation and OCT examination was repeated at all centers at months 3, 6 and 12. Due to the multicenter nature of this study, several brands of FA and SD-OCT devices were utilized to evaluate the study population. All prescheduled examinations were planned in the study groups on a monthly or bimonthly basis, except for FA. FA was repeated in the

follow-up depending on the physician's individual clinical decision—only when a new and unexpected clinical symptom has arisen. SD-OCT was used mainly for measurement of central macular thickness (CMT) values. CMT was defined as the mean thickness of the neurosensory retina in 1 mm diameter central foveal area as it was computed in all devices using automated integrated OCT mapping softwares.

Drug Administration All intravitreal injections were administered under sterile conditions. Following topical anesthesia and surface disinfection with 5% povidone-iodine, intravitreal 0.5 mL/0.1 mL ranibizumab or 2 mg/0.1 mL aflibercept were injected through the pars plana 3.5-4 mm posterior to the limbus *via* a 30-gauge needle. After the injection, topical 0.5% moxifloxacin (Vigamox; Alcon Laboratories, Inc., Fort Worth, Texas, USA) was prescribed 5 times a day for the following 2wk. Patients were examined on day 1 and at prescheduled monthly or bimonthly visits. Retreatment decisions had been obviously made by the primary attending retinal physician at each center-based on his or her clinical judgement , but major retreatment criteria had been generally accepted as visual decline >1 Snellen line compared to previous visit, presence or increase of sub- or intraretinal fluid on OCT, signs of reactivation of the CNVM such as newly detected macular hemorrhage. These retreatment criteria were strictly followed by all the investigators throughout the study period.

Statistical Analysis Following the data collection from all these tertiary centers , all BCVA values were converted into logMAR for statistical purposes. The data were evaluated for normality using the Kolmogorov-Smirnov test. As the distribution of the BCVA and CMT values were found to be normal, changes in these parameters between baseline and following time points were assessed with repeated measures test. Student's *t*-tests and repeated measures of ANOVA were preferred for inter-group and intra-group statistical analyses using SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA). An overall 5% type-1 error level was considered to be statistically significant.

RESULTS

Five hundred and forty-four patients (135 IVA; 409 IVR) diagnosed with nAMD were enrolled into the study according to our inclusion/exclusion criteria. The mean age was 73.74±8.6y (range 50-94y); 309 patients (56.8%) were men and 235 (43.2%) were women. Ninety-four eyes (15.7%) had been treated with either bevacizumab or ranibizumab before, while 450 eyes (82.7%) were defined as treatment naïve. In the total study population; 409 (75.2%) eyes were treated with 0.5 mg IVR, whereas 135 (24.8%) eyes were received 2 mg IVA therapy. According to the treatment regimen, 101 eyes (18.6%) were included into the 1+PRN arm, 443 eyes were enrolled into the 3+PRN arm of the study. Both arms were statistically comparable in the means of age, gender distribution, baseline visual acuity and CMT values. Baseline characteristics of the study population is summarized at Table 1. All patients had a minimum follow-up interval of 12mo. No systemic complication was reported in this one-year follow-up. Ocular complications were limited to punctate epitheliopathy (*n*=17, 3.1%), subconjunctival hemorrhage (*n*=48, 8.8%) and mild anterior chamber reaction (*n*=22, 4%). Severe complications such as endophthalmitis or retinal detachment were not encountered in any of the eyes during the study period.

Table 1 Baseline characteristics of both study arms were similar in means of age, gender distribution and mean values of visual acuity and CMT

Parameters	1+PRN group (*n*=101)	3+PRN group (*n*=443)	*P*
Age (y)	74.7±9.2	73.5±8.5	0.24
Gender (*n*; M/F)	79/56	230/179	0.71
Baseline CMT (μm)	407±134	398±138	0.54
Baseline BCVA (logMAR)	0.77±0.34	0.81±0.32	0.19

CMT: Central macular thickness; BCVA: Best corrected visual acuity.

Functional Results The mean baseline BCVA changed in 1+PRN group (*n*=101) of the total study population from 0.77±0.34 to 0.78±0.45 (month 3; *P*=0.79), 0.75±0.45 (month 6; *P*=0.58) and 0.75±0.44 (month 12; *P*=0.65). In the 3+PRN group (*n*=443), however, the mean BCVA increased significantly from 0.81±0.32 to 0.69±0.32 (month 3; *P*<0.001), 0.72±0.43 (month 6; *P*<0.001) and 0.76±0.46 (month 12; *P*=0.006). When the effect of treatment regimen on the visual results at all time points was analyzed, the 3+PRN group was found significantly superior over 1+PRN group in the follow-up (repeated measures; *P*=0.005; Figure 1). The most significant visual gain difference was found in favor of 3+PRN group at 3rd month visit (-0.01 *vs* 0.12; *P*<0.001). The mean numbers of injections (2.4 *vs* 4.4; *P*<0.01) and visits (6.4 *vs* 7.2; *P*<0.01) were significantly higher in the 3+PRN arm of the study population.

The total study population was divided then into two subgroups according to the type of anti-VEGF agent. Treatment regimens' visual outcomes were analyzed in IVR and IVA subgroups separately. In IVR subgroup (*n*=409), the mean BCVA of the 1+PRN arm (*n*=75) changed insignificantly from baseline 0.77±0.33 to 0.80±0.44 (month 3; *P*=0.60), 0.76±0.45 (month 6; *P*=0.68) and 0.77±0.44 (month 12; *P*=0.90). In 3+PRN arm (*n*=334), however, BCVA improved significantly from baseline value of 0.80±0.32 to 0.70±0.37 (month 3; *P*<0.001), 0.72±0.42 (month 6; *P*<0.001) and 0.76±0.46 at the final visit (month 12; *P*=0.09). The visual gain comparison

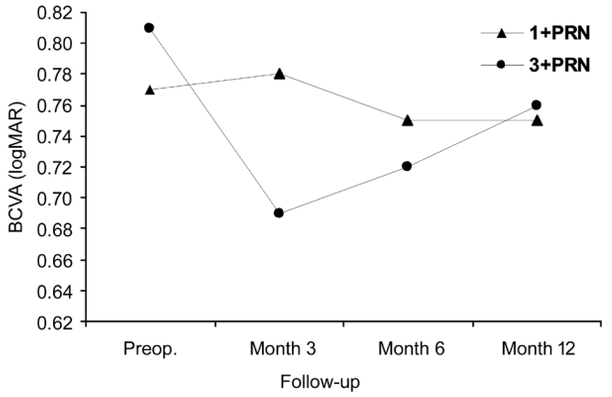

Figure 1 The comparison of visual gain between treatment groups in the total study population revealed a significant superiority of 3+PRN regimen over the 1+PRN approach (*P*=0.005).

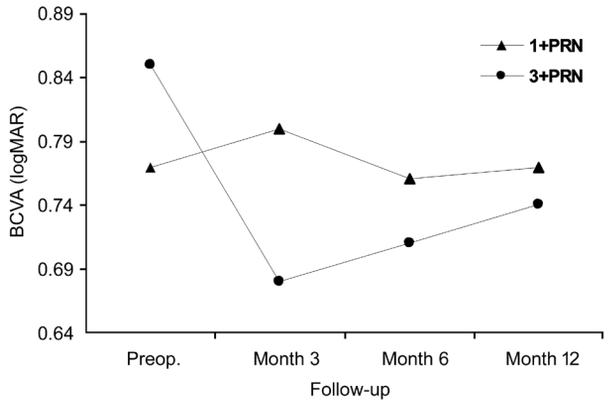

Figure 2 In IVR subgroup the superior visual results were significantly better in 3+PRN arm (*P*=0.003).

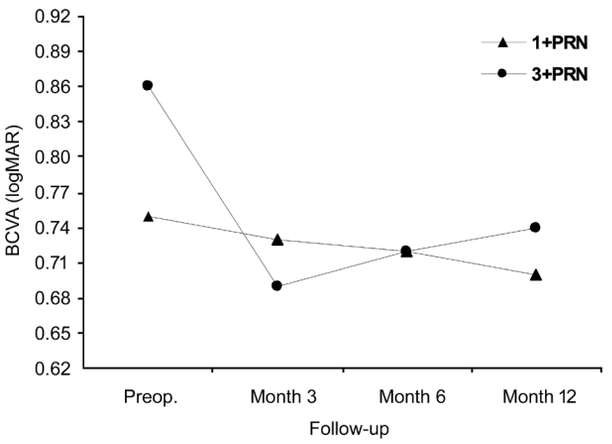

Figure 3 The visual results in IVA subgroup were in favor of 3+PRN arm, but did not reach statistical significance (*P*=0.068).

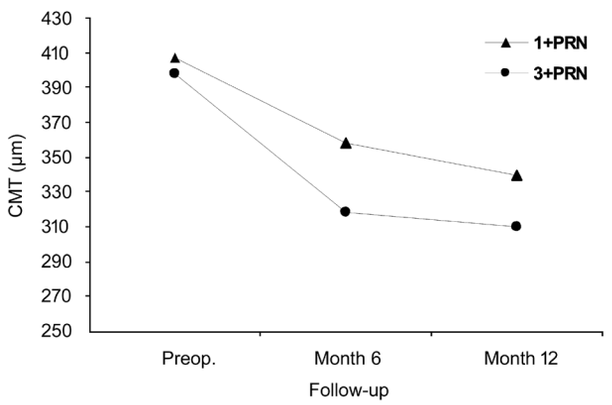

Figure 4 Both regimens in the total study population resulted in comparable anatomical gains in means of CMT reduction (*P*=0.08).

of different treatment arms in IVR subgroup revealed a statistically significant difference in favor of 3+PRN arm (repeated measures; *P*=0.003; Figure 2). The mean number of intravitreal treatments in one year was also found significantly higher in the 3+PRN (4.67 *vs* 2.97; *P*<0.001), although the mean numbers of visits demonstrated no significant difference in 1+PRN and 3+PRN arms (6.7 *vs* 7.1; *P*=0.19; respectively).

The visual outcome analyses in the IVA (*n*=135) subgroup revealed similar results in favor of 3+PRN regimen such as in the IVR subgroup. In 1+PRN arm (*n*=26), mean BCVA changed insignificantly from 0.75±0.36 (baseline) to 0.73±0.45 (month 3; *P*=0.70), 0.72±0.46 (month 6; *P*=0.69) and finally to 0.70±0.45 (month 12; *P*=0.47). The BCVA in 3+PRN arm (*n*=109) increased significantly from 0.85±0.33 (baseline) to 0.68±0.37 (month 3; *P*<0.001), 0.71±0.45 (month 6; *P*<0.001) and 0.74±0.49 (month 12; *P*=0.009). However, the comparison within the IVA subgroup revealed no significant difference between 3+PRN and 1+PRN arms (repeated measures; *P*=0.068; Figure 3). Although the mean number of visits in the study period were similar in 1+PRN and 3+PRN IVA arms (7.1 *vs* 7.5, respectively; *P*=0.47), the mean number of intravitreal administrations differed from each other significantly (2.4 *vs* 4.2, respectively; *P*<0.001).

Anatomical Outcomes The mean CMT value in the 1+PRN arm (*n*=101) of the total study population decreased significantly from baseline value of 407±134 μm to 358±111 μm (month 6; *P*<0.001) and to 340±111 μm (month 12; *P*<0.001) at the final visit. Likewise, in the 3+PRN arm the mean CMT was reduced significantly from 398±138 μm to 318±103 μm (month 6; *P*<0.001) and finally to 310±101 μm (month 12; *P*<0.001). The statistical between-group comparison in aspect of anatomical gain showed that there was no significant difference between these two regimens (repeated measures; *P*=0.08; Figure 4).

In the sub-analyses of anti-VEGF agent based subgroups; the mean CMT in IVR 1+PRN arm (*n*=75) declined significantly from 395±124 μm (baseline) to 351±112 μm (month 6; *P*<0.001) and to 334±113 μm (month12; *P*<0.001). Meanwhile, the baseline CMT in IVR 3+PRN arm (*n*=334) decreased also significantly from 398±135 μm (baseline) to 315±100 μm (month 6; *P*<0.001) and to 307±103 μm (month 12; *P*<0.001). The CMT gain analyses between 1+PRN and 3+PRN arms within IVR subgroup revealed no significant difference (repeated measures; *P*=0.14; Figure 5).

In IVA subgroup; the mean CMT value of 1+PRN arm (n=26) was reduced significantly from 444±155 μm (baseline) to 380±107 μm (month 6; P=0.019) and finally to 356±136 μm (month 12; P=0.018). In 3+PRN arm (n=109) the CMT declined very significantly from 421±144 μm (baseline) to 330±112 μm (month 6; P<0.001) and to 321±98 μm (month 12; P<0.001). The anatomical gain comparison between 1+PRN and 3+PRN arms within IVA subgroup was analyzed and no statistical difference was found through the study period regarding the treatment regimen (repeated measures; P=0.47; Figure 6).

The visual prognosis of study population was evaluated also in means of visual gain and loss percentages at month 12. The ratio of visual gain ≥3 Snellen lines (+15 ETDRS letters equivalent) at the final visit was found in 1+PRN and 3+PRN arms as 11.9% and 15.6% (P=0.34), respectively. In IVR subgroup analysis, the visual improvement (≥3 Snellen lines) ratios in 1+PRN and 3+PRN arms revealed no significant difference (12% *vs* 13.8%, respectively; P=0.67). The same comparison in IVA group pointed to a slight superiorty of 3+PRN (21.1%) over 1+PRN (11.5%) regimen, although it did not reach a statistically significant level (P=0.27). The visual loss ≥1 Snellen line (-5 ETDRS letters equivalent) was found in similar ratios in 1+PRN and 3+PRN arms of the total study population (10.9% *vs* 11.9%, respectively, P=0.96). Hence, In IVA and IVR subgroups both arms visual loss analysis showed comparable ratios at month 12 (8.3% *vs* 6.6%, 13.3% *vs* 12.1%) respectively.

DISCUSSION

The AMD is one of the major etiologies for legal blindness in the developed countries over a certain age and this status is increasing exponentially with the overall life expectancy and crowding risk factors. The mainstay therapy of this devastating disease remains still anti-VEGF treatment options. Pivotal trials for administrative approvals recommended several regimens such as fixed monthly, a PRN approach following 3-5 initial loading doses or a priori as needed regimen. All collective data suggest a strict follow-up and prompt treatment since a delay of therapeutic intervention might cause irreversible destruction of foveal microstructure leading to a permanent visual impairment[9]. Despite this well-known fact, heavy treatment burden for both the patients and clinicians inhibit an ideal therapeutic follow-up. Several real life based studies[10-11] reported already this deviation of the results in real settings from the data of clinical trials conducted under controlled ideal circumstances. With the current study we aimed to review our own real life nAMD treatment outcomes in Turkish population and evaluate the effect of initial loading phase-based on the data driving from the nine tertiary reference centers of the most populated cities (Istanbul, Kocaeli) of Turkey.

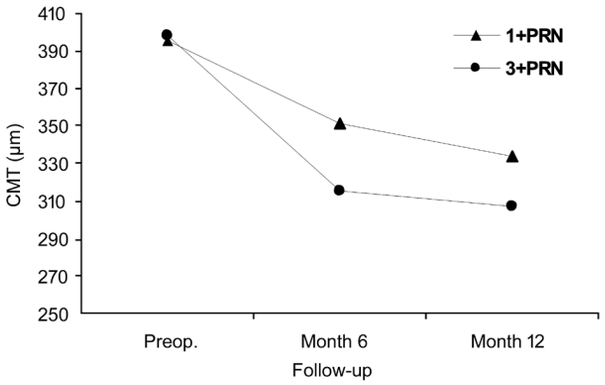

Figure 5 The CMT reduction was found statistically comparable in both regimen arms of the IVR group (P=0.14).

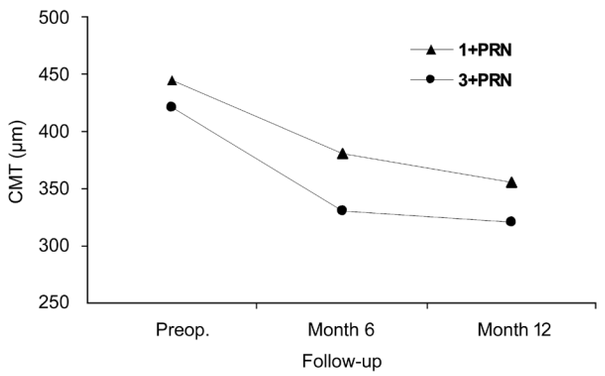

Figure 6 The CMT reduction in the IVA subgroup was similar in both arms (P=0.47).

Our anatomical results demonstrated no significant difference in mean of CMT gains between 1+PRN and 3+PRN regimen arms in the total study population and both-IVR or IVA-subgroups, although significantly more intravitreal injections had been administrated in 3+PRN arms than in 1+PRN arms of the total study population, IVR and IVA subgroups (4.4 *vs* 2.4; 4.67 *vs* 2.97; 4.2 *vs* 2.4, respectively). This finding might be related to the fact that unlike the greater and continuous CMT reductions in *e.g.* diabetic macular edema treatment outcomes, CMT value changes in nAMD remain within a limited range due to the presence of the underlying and despite the treatment persisting CNVM. Our mean CMT values remained almost unchanged after the 6th month visit where all groups have obviously reached a plateau in anatomical gain. We found this finding consistent with the previous reports. In the two-year results of HARBOR study Ho *et al*[12] reported a rapid reduction of CMT at day 7 continuing through month 3 and the CMT values sustained in the further 24mo follow-up to the same extend regardless of treatment or dosing regimen. Although the most frequent anatomical retreatment criterion was accepted as an increase in CMT[13], an analysis revealed the fact that CMT does not correlate with visual function in AMD since this correlation between function and structure is lost as early as month 3 of the follow-up[14].

In real life studies such as the LUMINOUS study[15], the average number of injections in year 1 was reported as 4.3, 5.5, 4.7, and 5.0 in Germany, the Netherlands, Sweden, and Belgium, respectively. Later, Holz *et al*[16] again declared in a multi-country real-life experience study the mean injection numbers as 5.0 and 2.2 in 1st and 2nd year respectively, where the highest scores were achieved by the UK sites. Besides, the Writing Committee for the UK Age-Related Macular Degeneration EMR Users Group reported in their first report three years outcome from the UK and presented their mean injection numbers in year 1, 2 and 3 as 5, 4 and 4, respectively[17]. Hykin *et al*[18] concluded in a further report that high monitoring and treatment rates in the UK resulted in better visual acuity outcomes compared with other countries. In a real life single center study from Turkey, Cebeci *et al*[19] published their two years results and reported a mean number of treatments as 5.8 and 4.2 in the first and second year, respectively. Küçük *et al*[20] also found similar injection numbers (4.5 in year 1 and 1.6 in year 2) in their retrospective report regarding their single center IVR treatment results in AMD. Similar to these results, our mean anti-VEGF administration number in the 3+PRN arm of the total study population was 4.4 and proved itself also comparable with the first year data of the above mentioned developed countries. In the 1+PRN arm, however, the low mean injection number (2.4, in total population) indicated to a sub-optimal treatment of those patients, even under real life circumstances. This fact has led to a significantly lower final visual gain in 1+PRN arm of the total study group as well as in IVR and IVA subgroups.

The prospective single center PronTo study[21] advocated for the efficacy of an OCT based 3+PRN ranibizumab regimen against the earlier recommended fixed monthly dosing approach. In this study, Lalwani *et al*[21] reported a visual gain of 11.1 letters comparable to ANCHOR and MARINA trials with a significantly less mean number of injections (9.9 *vs* 24 each) in their 24mo follow-up. Previously, in the first year results of the same study, Fung *et al*[22] reported a visual improvement of 9.3 letters (approximately 1.8 Snellen lines), a visual gain ≥15 letters in 35 % of the patients and a mean CMT reduction of 178 μm compared to baseline. They apparently achieved these results with a mean number of 5.6 injections at the end of 12mo. These anatomical and functional outcomes demonstrate a clear superiority over the results of our 3+PRN arm (178 *vs* 84 μm; 35% *vs* 15.6%). We believe, the prospective PronTo studies' strict monthly monitoring regimen combined with "zero tolerance" retreatment criteria might contribute to this significant difference. In contrast to their 12 monthly visits in one year, the mean visit number in our 3+PRN arm was only 7.2, exposing our deficiency of close follow-up in even a 3+PRN regimen.

There are several studies in the literature questioning the necessity of the initial loading phase in anti-VEGF therapy of nAMD. Menon *et al*[23] compared in their prospective randomized BeMOc trial loading and no loading regimens of intravitreal 1.25 mg bevacizumab and concluded that gain in visual outcome following a loading regimen was not as impressive as expected but still clinically justified. Additionally, the loading phase did not increase the first year's injection number significantly. Earlier reports also emphasized the importance of fixed initial loading doses. Arias *et al*[24] found in a non-randomized study with small sample size that a loading phase with bevacizumab resulted in better visual outcome compared to no loading at the end of 6mo follow-up. In a retrospective, non-randomized study Gupta *et al*[25] compared loading and non-loading IVR groups and reported the superiority of their loading group over the non-loading in the means of visual outcome. On the other hand, two studies claimed that a loading phase might not be essential in the AMD treatment. In the CATT trial, the PRN arms of both bevacizumab and ranibizumab groups had no initial loading phase but the investigators found non-inferior final visual outcomes compared to the fixed monthly regimen[26]. Later, El-Mollayess *et al*[27] reported that there was no significant difference in means of visual gain between a fixed monthly regimen of bevacizumab and a priori PRN regimen without any loading doses. In both of these studies there was apparently a strict follow-up and low threshold retreatment protocol based on OCT findings. In our study population, however, loading phase enhanced visual outcome in all subgroups significantly, particularly due to the fact that we treated the patients in the 1+PRN groups in a suboptimal dosing.

In conclusion, this retrospective study was the first national broad-based nAMD research conducted by clinicians from nine most referred clinical centers, reflecting the real life treatment results in Turkish population. The limitations of this study such as clinician based retreatment criteria on a PRN regimen or irregular visits were deriving from its multi-centered and retrospective nature; we tried to eliminate these limitations and selection bias by strictly following our exclusion-inclusion and retreatment criteria and excluding the non-copying patient data from the study. Following our first report[28], this current study gave us all the investigators a critical insight into our treatment preferences and its consequences within an earlier time interval. Although both regimens resulted in similar anatomical outcomes in means of CMT, the 3+PRN arm clearly demonstrated—regardless of the anti-VEGF agent-the vital role of three initial consecutive doses for desirable visual outcomes. Hence, all the investigators were convinced from the need of a re-adjustment of their clinical approach and the importance of the initial loading phase in nAMD treatment.

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