Graefe's Archive for Clinical and Experimental Ophthalmology
https://doi.org/10.1007/s00417-018-3974-0

RETINAL DISORDERS

The reactivation time in the treatment of AMD: a forgotten key parameter?

J. P. Real[1] · J. D Luna[2] · S. D. Palma[1]

Received: 19 February 2018 / Revised: 20 March 2018 / Accepted: 26 March 2018

Abstract

Objective Summarize and compare the available evidence on the reactivation times in patients with age-related macular degeneration treated with Ranibizumab (RNB).

Method Systematic review of studies that reported the reactivation time of patients (direct method) or the number of injections received in a certain period of follow-up (indirect method).

Results Only 18 of 89 selected studies reported the average reactivation time of patients in a manifest form, without the need of any calculation. The average calculated, weighted reactivation time was 101.8 days with the direct method and 99.8 days in the indirect method (84 studies included). With both methods, it was found that the average reactivation time of the RCTs was between 2 and 3 weeks less than the average time identified in the observational studies. These differences are also reflected in the clinical results, there being a correlation between the number of doses received and the change in BCVA. The analysis of 11 comparative studies showed a difference in reactivation times between patients treated with RNB or Bevacizumab (BVZ).

Conclusion There are few direct studies of reactivation time, but calculation from the PRN dose number turns out to be a good approximation for retrospective study of the variable. The use of the PRN, with criteria not based on optical coherence tomography scans, delays the application of doses between 2 or 3 weeks, and patients suffer loss of clinical benefits. RNB enables patients to receive less injections than BVZ throughout treatment.

Keywords Time-to-treatment · Ranibizumab · Macular degeneration · Angiogenesis inhibitors/therapeutic use

Introduction

Age-related macular degeneration (AMD) is the leading cause of severe visual loss in the developed world [1]. The incidence and prevalence of AMD are increasing as the population ages and life expectancy lengthens [2, 3]. The current treatment for neovascular AMD is based on the use of therapeutic entities that specifically inhibit vascular endothelial growth factor (VEGF) by blocking the protein. Ranibizumab (RNB) was the first anti-VEGF drug to receive approval by regulatory agencies and is one of the most studied within the scientific literature.

The use of anti-VEGF therapy was initially approved in a monthly injection scheme, based on the results of phase III multicentric clinical studies that served for the approval of the drug [4, 5]. Despite the excellent results shown, there was a need to find new schemes that would reduce costs and/or burden on the patient. In other words, it was necessary to achieve comparable visual results with fewer injections and/or visits [6, 7].

Since approval of the monthly scheme, many schemes have been proposed and taken to clinical practice. However, the logic why certain monitoring and reinjection criteria were used have not been entirely clear, and in many cases, the schemes are difficult to carry out in routine clinical practice [8, 9]. The determination of an optimal dosage scheme for anti-VEGF therapy in neovascular AMD should be based on

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00417-018-3974-0) contains supplementary material, which is available to authorized users.

✉ S. D. Palma
http://www.fcq.unc.edu.ar

[1] Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA), CONICET and Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina

[2] Vitreo-Retinal Department, Centro Privado de Ojos Romagosa SA - Fundación VER, Deán Funes 429/432, Córdoba, Argentina

Published online: 19 April 2018

scientific knowledge regarding the nature of the disease and the effects of the drugs on it.

The use of anti-VEGF drugs does not solve the underlying cause responsible for the disease but only temporarily eliminates the excessive presence of VEGF in the affected eye. When the drug, due to pharmacokinetic issues, is no longer present at the site of action, VEGF will begin to accumulate until it again reaches excess [10]. Therefore, the application of RNB injections at a time before the onset of recurrence can prevent choroidal neovascularization injury, protecting the retina from additional damage due to constant recurrences and improving long-term prognosis. Although this concept is achieved with monthly injections, the results obtained with the pro re nata scheme (PRN) show that monthly application would be excessive [11]. Knowing the times of reactivation, therefore, seems to be an important topic in the design of a strategy with a pathophysiology basis for the treatment of AMD.

Survival analysis refers to the set of techniques that allow studying the variable "time until an event occurs" and its dependence on other possible explanatory variables. The parametric approach of the survival analysis is to accept that a certain type of parametric distribution function serves as a model for the evolution of the survival function [12–14]. This approach characterizes the survival function as a function dependent on the accumulated risk of the occurrence of an event ($H(t)$) and models the variable with an exponential function when it considers that the instantaneous risk of occurrence of the event is constant ($h(t)$) [15].

$$\begin{aligned} P &= 1-S = 1-e^{-H(t)} \\ h(t) &= constant = \lambda \\ H(\mathrm{t}) &= \lambda * t \\ S(\mathrm{t}) &= e^{-H(t)} = e^{-\lambda t} \end{aligned}$$

where

- *P is the cumulative probability of suffering the event at time t.*
- *S is the survival function at that time.*
- *H(t) is the accumulated risk rate of the event happening at time t.*
- *h(t) is the risk or instantaneous risk function at time t.*

On the other hand, the variables that describe the number of times an event is repeated in a predetermined time present Poisson type distributions. Parameters of this distribution are directly related to the underlying exponential variable, that is, with the variable that measures the time between two successive events [16]. If then we consider that in a PRN scheme, the maintenance doses are applied as long as there is a new reactivation, we can affirm that there is a relationship between the number of times the reactivations occur in a given time and the time necessary for the occurrence of a only reactivation (Table 1).

Therefore, it is possible to investigate the reactivation time in two ways:

1. Directly from the studies that specifically reported the average time of reactivation
2. Indirectly through the works that reported the number of maintenance doses (PRN scheme) applied in a given time

The aim of this work was to identify, summarize, and compare the available evidence on the reactivation times in patients with AMD treated with RNB using both methods. We also compare in this study the results obtained in randomized clinical trials (RCTs) with those obtained in observational studies, and we analyze the possible differences with other anti-VEGF drugs.

Methods

The methodology applied to carry out the meta-analysis was adapted to the recommendations of the Cochrane association [17].

Eligibility criteria

We selected those studies (1) that treated patients diagnosed with neovascular AMD, (2) that at least one of the treatment branches received Ranibizumab in a PRN regimen in monotherapy, (3) that reported the reactivation time of patients or the number of injections received in a given follow-up period, and (4) of those who presented a prospective or retrospective longitudinal design and (5) who presented a follow-up of the patients at least 1 year from the initial dose.

We excluded works that studied patients with choroidal neovascularization secondary to diseases other than exudative AMD or that were made from the results obtained by other studies. Finally, those studies in which it was not possible to access the full text or a sufficiently informative summary were also excluded. No language restrictions were applied. The search was restricted to articles published from January 1, 2009, to December 31, 2014, the 5 years prior to the start of this work.

Search strategy

The search strategy was based on the combination of terms Mesh (Medical Subject Headings) and keywords related to each term, combined by the boolean operators AND, OR, and NOT.

The keywords of interest used to identify terms were "Age-related Macular Degeneration," "Anti-VEGF," "Ranibizumab,"

Table 1 Relation between the probabilistic distributions of the reactivation time and the number of maintenance doses

Distribution Random variable	Exponential Time between successive events	Poisson Number of events at a time *t*
Density function	$=\lambda * e^{-\lambda * t}$	$=\frac{(\lambda * t)^n * e^{-\lambda * t}}{n!}$
Mean	$\frac{1}{\lambda}$	$\lambda * t$
Cumulative Probability	$=1-e^{-\lambda * t}$	$\sum Pn$ (Pn = partial probabilities)
Example	Reactivation time	Number of maintenance doses

"variable dosing," "treatment intervals," "time to reinjection," "Time-to-Treatment," "flexible retreatment," and "pro re nata regimen." Also, the additional requirement that the articles correspond to prospective or retrospective longitudinal clinical studies was taken into account. Therefore, terms and keywords related to them were introduced.

The databases consulted for the identification of the studies were PubMed (www.ncbi.nlm.nih.gov) and SCOPUS (www.scopus.com).

The strategies (detailed in Supplementary S1) were modified to meet the needs of each database and were complemented by manual searches of the bibliographies of other reviews and searches of the registry for clinical trials (clinicaltrials.gov).

Selection of studies and extraction of data

The titles and abstracts of the identified studies were reviewed independently by two reviewers (JPR and JDL) using an instrument specially designed for the selection of articles (Supplement S2).

In the event that there were discrepancies between the two reviewers, a third investigator was appointed who arbitrated and made the decision (SDP). The complete works of the pertinent studies were obtained for detailed evaluation and extraction of the relevant information by the same two reviewers (JPR and JDL).

The primary outcome of interest defined for this review was reactivation time in those studies that specifically reported it and the number of maintenance doses as well as follow-up time in studies used for the indirect calculation.

$$No.of\ \ maintenance\ \ doses = \lambda \times follow\ up\ time$$

$$mean\ \ reactivation\ \ time = \frac{1}{\lambda} = \frac{follow\ up\ time}{No.of\ \ maintenance\ \ doses}$$

Mean change in visual acuity between baseline and 1 year was included as a secondary finding.

Statistical analysis

This systematic review is defined as a meta-analysis since it performs a quantitative analysis of the results obtaining an estimate of the variables under study from weighting of the results of the different studies that compose it. To do this, the following formula applies

$$\overline{T} = \frac{\sum_{i=1}^{k} w_i T_i}{\sum_{i=1}^{k} w_i}$$

where *Ti* is the effect measured in study *i* and *Wi* is the weight that is assigned to said study *i*. The weighting of the studies was based on the number of participants included in the study.

The meta-analysis was performed using the Infostat package (2016 version) and software from the Cochrane Collaboration RevMan 5.3. The difference in standard means (SMD) was used for the analysis of the comparative studies. Statistical heterogeneity between the studies was assessed by the Q-statistic and quantified by the I2 statistic. Both a fixed-effect model and a random-effects model were used. In the absence of heterogeneity between the groups, the fixed-effect model was used to provide concordant results. The random effects model was used only when heterogeneity was significant ($P < 0.1$ and $I2 > 50\%$). The level of significance was set at 0.05.

Results

Identification and selection

A total of 699 articles were identified by adding both databases. The literature search process is summarized in Fig. 1.

Analysis of the remaining studies included

A total of 89 different studies were included, 15 RCTs, 22 non-blind prospective studies (three comparative and 19

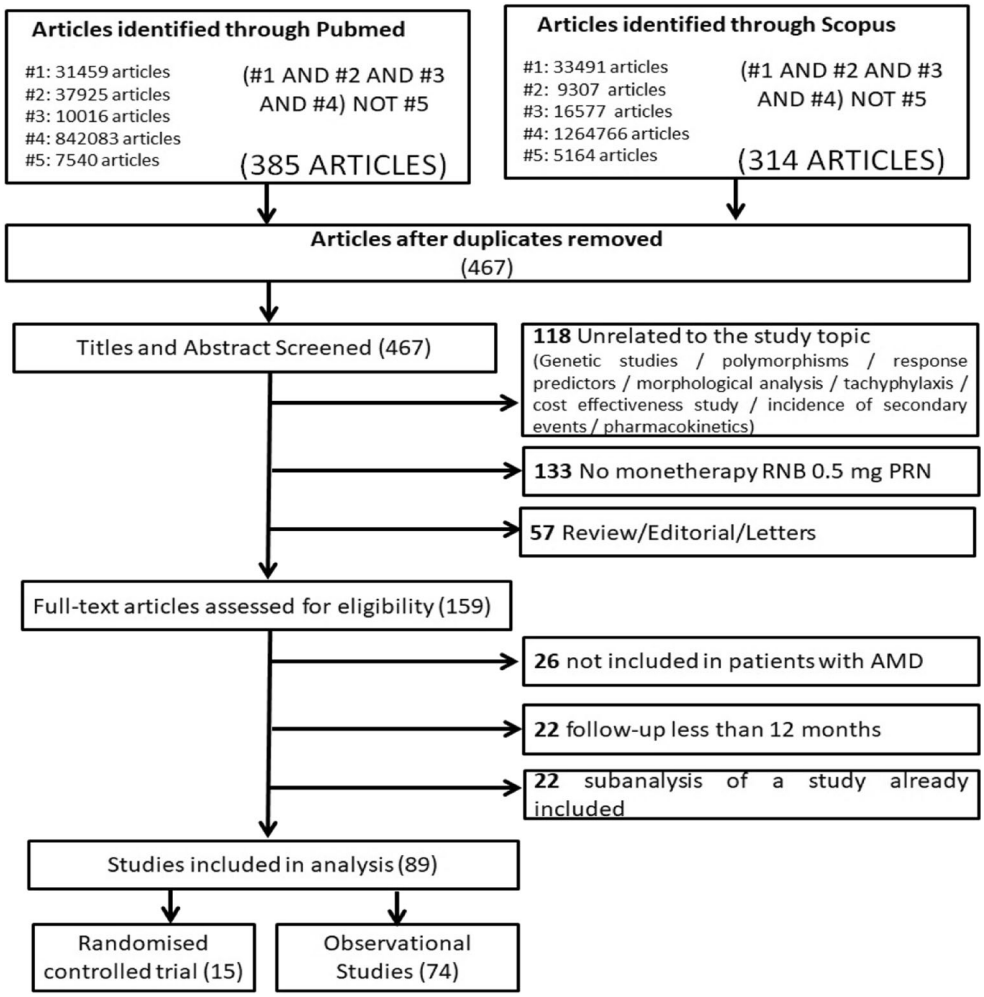

Fig. 1 Flow diagram of literature retrieval. Nos. 1, 2, 3, 4, and 5 are the components of the search strategy detailed in Supplementary S1

non-comparative), and 52 retrospective studies (10 comparative and 42 non-comparative). The global analysis included a total of 25,279 patients, 61% of them females, average of 77.7 years of age, and 53.8 ETDRs letters of visual acuity in the baseline. The main difference between observational studies and RCT was observed in visual acuity at baseline (57 and 53.6 ETDRS letters, respectively).

Analysis of the studies according to the reactivation times

Among the 89 studies included, only 18 (6055 patients) reported the average reactivation time of the patients in a manifest form, without the need of any calculation, while 84 studies (23,680 patients) required calculation of the average reactivation time in an indirect manner. The average calculated weighted reactivation time was 101.8 days with the direct method and 99.8 days in the indirect method. The main difference between the two analyses can be observed in the standard deviations, which was 33 days (range 40 to 168 days) for the direct method and 51 days (range 54 to 286 days) in the indirect method.

When the analysis was subdivided according to the study methodology, it was found, with both methods, that the average reactivation time of the RCTs is less than the average time identified in the observational studies. When only studies that reported directly are considered, an approximate difference of 27 days is observed between the times reported by the observational studies (102.9 days) and the clinical trials (76.05 days) (Table 2).

When the analysis was made from the works with which the reactivation time was calculated indirectly, the difference is extended to 21 days (101.7 days in observational studies and 80.5 days in clinical trials) (Table 2).

The daily risk rate (λ) of reactivation calculated from the observational studies is equal to 0.0098 (1/102.0 days) reactivations/days with both methods. When the analysis is performed from the RCTs, the risk rate increases to 0.0131 (1/76.05 days) or 0.0124 (1/80.5 days) reactivations/days, depending on whether the method considered is direct or indirect, respectively.

Table 2 Results of the calculation of the average time of reactivation

Direct method: studies that observed the reactivation time

Type of study	No. of patients	Weighting		λ	Reactivation time (days)
Observational studies	5587	93%		0.0097	102.92
RCT	408	7%		0.0131	76.05
Totals	6055	100%		0.0099	101.10
Indirect method: studies that reported the number of PRN maintenance doses in a time *t*					
Type of study	No. of patients	No. of PRN injection	Time *T* (days)	λ	Reactivation time (days)
Observational studies	20,629	5.17	514.1	0.0098	101.7
RCT	1599	4.86	378.8	0.0124	80.56
Totals	22,228	5.15	504.4	0.01	99.82

The details of the studies included in the calculation are detailed in Supplementary S3

PRN pro re nata, *Time T* follow-up time to patients, λ daily risk rate of reactivation

Clinical results

Of the 89 included studies, 77 reported the visual acuity changes experienced by the patients throughout the treatment. The analysis showed that this variable has a significant direct correlation with the number of doses received and the change in AV experienced between the end of the loading phase and the end of the follow-up at 12 months (Pearson correlation: Coefficient 0.3, p value < 0.01). In other words, the larger the inter-injection interval or the smaller the number of injections received by the patient, the lower the probability that the improvements achieved in the loading phase will be sustained over time.

These differences are clearly reflected when comparing the results of clinical trials and observational studies (Fig. 2). Although in both cohorts, there is a decrease in visual acuity throughout the maintenance phase, this decline in visual acuity is more pronounced in observational studies where the frequency between injections was lower.

Comparative studies

Among the 28 comparative studies, nine different types of comparisons are observed, highlighting those that compared the use of Ranibizumab with Bevacizumab (11 studies).

LUCAS [18] and IVAN TRIAL [19] were excluded in the previous analyses because, although they use a variable scheme, they do not use a strict PRN scheme (LUCAS uses TAE and IVAN uses a scheme of three injections after each reactivation).

It is interesting to observe the existence of significant differences in the number of doses received by patients after 1 year of follow-up since the first injection. The global analysis, including all the works, indicates that there would be no significant difference between the drugs ($p = 0.13$), although a high heterogeneity is denoted (I2 = 92%). When, on the other hand, the focus is only on studies that have a rigorous follow-up (RCTs), it is clear that patients who use RNB require significantly fewer injections (7.1 vs 6.2 injections in patients treated with BVZ and RNB, respectively) ($p < 0.0001$ Fig. 3). The heterogeneity observed in the studies that were compared disappears (I2 = 0%, $p = 0.44$) when we exclude the small and difficult to compare study conducted by Subramanian et al., which only included seven patients with RNB.

When only RCTs are analyzed, a difference in reactivation times is observed between patients treated with RNB or BVZ. The time difference between the patients treated with some of the drugs mentioned above was 15 days (85 vs 70 days).

Discussion

This review was specifically designed to gather and analyze information regarding the reactivation times experienced by patients with AMD treated with RNB. The results show that in spite of the importance of the study of the reactivation time, there are few works that report it specifically as relevant data. Although there are some works designed specifically for this purpose, they are smaller in extent.

Within the studies found, it is worth highlighting those that measured the intraindividual reactivation intervals [20, 21]. These studies show that although there is variability between the reactivation times of patients, there is also a high consistency between the times of successive reactivations suffered by the same patient with very small standard deviations. In other words, the reactivation time would be unique for each patient, therefore constant and predictable for each individual.

A regular rhythm of recurrences is not surprising if it is assumed that there is a continuous and stable rate of production (and elimination) of VEGF that overproduces to achieve excessive (and pathological) levels of VEGF in the retina. It

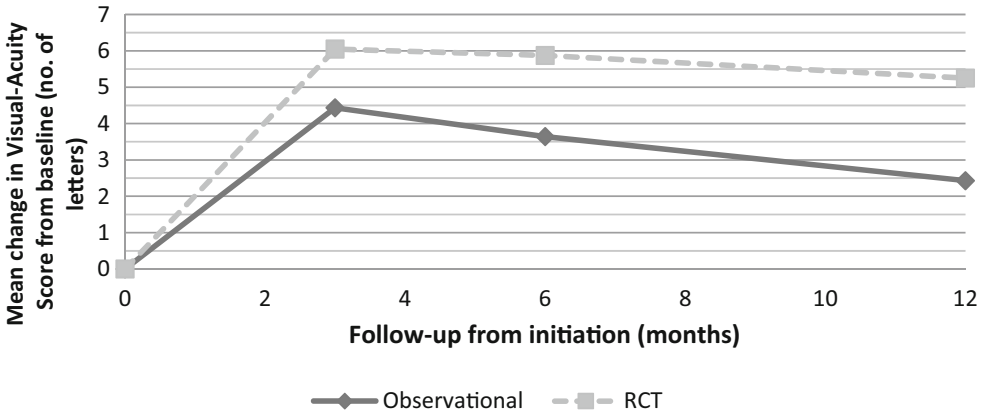

Fig. 2 Average change in visual acuity of patients with neovascular age-related macular degeneration treated in the context of observational studies and randomized clinical trials (RCTs)

would also be necessary to assume the existence of stable individual pharmacokinetics [10]. Muether et al. [22] studied VEGF suppression times (VTS) from the analysis of aqueous humor samples taken from patients treated with RNB. The authors observed that after an injection, the basal concentration of VEGF-A measured in the aqueous humor sample before the dose is completely suppressed. After a while, the levels of VEGF-A increase until reaching values similar to those found before the application of the doses. This pattern of suppression/reactivation was repeated in all the groups analyzed from samples of individual patients, the times of suppression being different between patients but very stable intra-individually. Finding a temporopathological correlation between the individual levels of macular concentration of VEGF and intraocular dynamics of the antibody that would also be unique for each patient.

On the basis of these studies, it is consistent to think that patients present an intrinsic time of reactivation and that the variability observed throughout the different studies has to do with the differences between patients. *Studying the variability of reactivation time will therefore allow us to know the way in which patients are distributed according to their intrinsic rhythm.* With this objective in mind, it was decided to include all the studies that described reactivation times directly or indirectly. The indirect method allowed us to increase the sample of patients studied. The similarity observed between the average time calculated with the direct method (101.8 days) and indirect method (99.8 days) gives confidence to the chosen method.

	Bevacizumab			Ranibizumab				Mean Difference	Mean Difference
Study o Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random; 95% CI	IV, Random; 95% CI
Observational									
Fong	4.41	2.41	324	6.23	2.16	128	7.80%	-1.82 [-2.28, -1.36]	
Biswas 2011	4.3	3	50	5.6	3	54	6.30%	-1.30 [-2.45, -0.15]	
Bellerive	4.75	4	139	4.92	4	45	5.80%	-0.17 [-1.51, 1.17]	
Carneiro	5.92	2.1	85	5.97	2.4	60	7.30%	-0.05 [-0.80, 0.70]	
Ozkaya 2013	4.8	1.2	79	4.7	1.4	74	7.90%	0.10 [-0.31, 0.51]	
Real 2013	4.71	1.45	52	2.98	0.55	44	7.90%	1.73 [1.30, 2.16]	
Subtotal (95% CI)			729			405	43.1%	-0.23 [-1.48, 1.01]	
Heterogeneity: Tau² = 2.24; Chi² = 129.35, df = 5 (P < 0.00001); I² = 96%									
Test for overall effect: Z = 0.37 (P = 0.71)									
ECA									
GEFAL 2013	6.8	2.7	191	6.5	2.4	183	7.70%	0.30 [-0.22, 0.82]	
MANTA 2013	6.1	2.8	154	5.8	2.7	163	7.60%	0.30 [-0.31, 0.91]	
CATT SWITCH	5.8	4.4	122	5	3.8	130	6.70%	0.80 [-0.22, 1.82]	
CATT PRN	7.7	3.5	300	6.9	3	298	7.70%	0.80 [0.28, 1.32]	
Scholler 2014	5.8	2.28	26	5	1.67	29	6.50%	0.80 [-0.27, 1.87]	
LUCAS 2014	8.9	2.2	213	8	2.3	218	7.90%	0.90 [0.48, 1.32]	
IVAN TRIAL	7	4	274	6	4	287	7.50%	1.00 [0.34, 1.66]	
Subramanian 2010	7.6	2.58	15	3.85	1.21	7	5.30%	3.75 [2.17, 5.33]	
Subtotal (95% CI)			1295			1315	56.9%	0.84 [0.43, 1.25]	
Heterogeneity: Tau² = 0.20; Chi² = 19.89, df = 7 (P = 0.006); I² = 65%									
Test for overall effect: Z = 4.05 (P < 0.0001)									
Total (95% CI)			2024			1720	100%	0.46 [-0.14, 1.07]	
Heterogeneity: Tau² = 1.16; Chi² = 169.72, df = 13 (P < 0.00001); I² = 92%									
Test for overall effect: Z = 1.50 (P = 0.13)									
Test for subgroup differences: Chi² = 2.59, df = 1 (P = 0.11), I² = 61.4%									

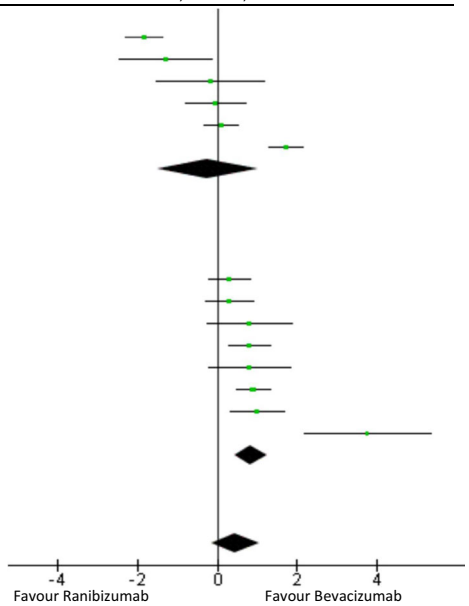

Fig. 3 Forest plots of the average number of intravitreal injections applied after 1 year of treatment in the direct comparative studies between Ranibizumab 0.50 mg and Bevacizumab 1.25 mg. Graph obtained using RevMan version 5.3

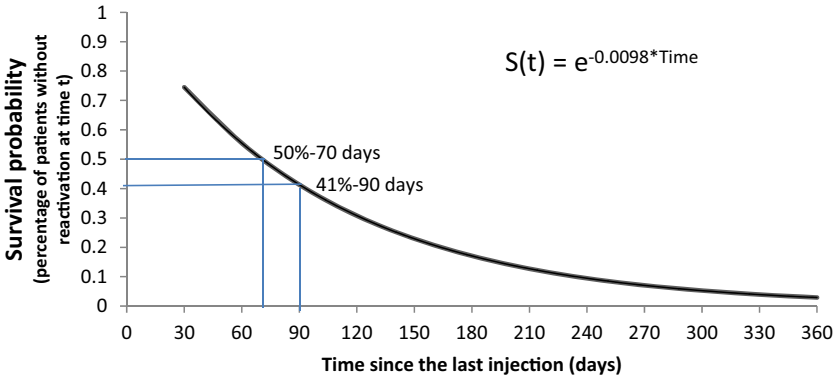

Fig. 4 Function of survival of the functional reactivation time. It represents the percentage of patients who have not yet reactivated at any given time. Example: at 90 days, 41% of patients did not suffer a reactivation

Evaluation of the average reactivation time requires correct interpretation since it can be misleading. One might think that 50% of patients do not reactivate until 102 days after the last dose; however, this is incorrect. The data that really matters is the risk function (λ), from which we can estimate the accumulated probabilities of patients. According to the estimated risk rate (0.0098), 50% of patients present a reactivation before 70 days after the last injection (Fig. 4).

Muether et al. [22] studied this time and observed that recurrences always follow the same pattern of behavior: *1st VTS occurs*, then the so-called *morphological recurrence* occurs (which is one that can be observed through optical coherence tomography (OCT) or complementary studies) and finally the *functional recurrence* occurs, which is expressed at the clinical level by changes in the visual acuity of the patient fundamentally. That is, we can distinguish at least two different reactivation times, which differ in time from one another, with the morphological being before the functional.

In clinical practice, in a context of increasing demand and limited resources, it is common that there is a sub-use of OCT so that ophthalmologists can resort to the use of clinical criteria to determine the need for reinjection [23]. In contrast, in clinical trials, the PRN scheme is carried out with strict follow-up with high sensitivity reactivation criteria (presence of any subretinal or intra-retinal fluid on OCT or leakage on FA) [24]. Taking this into account, we could consider that while clinical trials measure morphological reactivation times, it could be possible that observational studies do the same for functional reactivation. If we consider this as true, the results of the review would indicate that the morphological reactivation occurs on average at 76 or 80 days after the last injection, while functional reactivation occurs on average at 102 days. While these times differ from those reported by Muether [22] (93.7 days morphological reactivation and 114.3 functional reactivation), the difference between the times of occurrence of both reactivations is closely related. This means that approximately 21 to 27 days must elapse before a morphological reactivation can be expressed in a functional form. These times are coincident with what was reported in previous studies [25], where we had calculated the "rate of vision loss in neovascular age-related macular degeneration." In the first 3 weeks after reactivation, there was an average loss of five letters, a characteristic clinical benchmark to define the need for reinjection.

The available scientific evidence reveals that patients who suffer VA losses after a reactivation only recover a part of this loss when receiving a maintenance RNB injection [26]. Therefore, in the design of a new treatment scheme, this type of loss should be avoided in order to preserve clinical improvements obtained in the

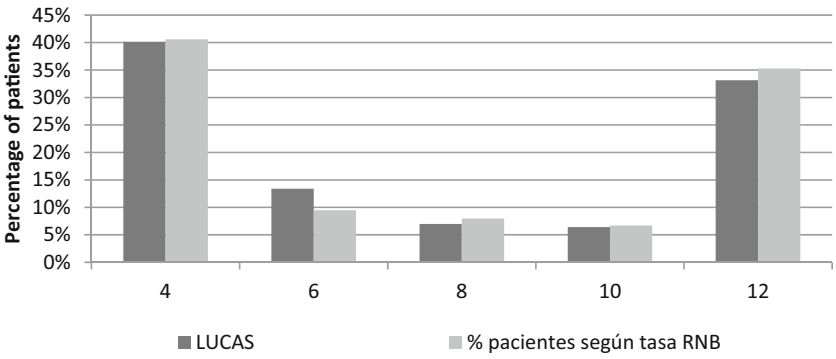

Fig. 5 Distribution of the calculated reactivation frequency and that observed in the patients who were injected in the Treat and Extend scheme in the LUCAS study

loading phase over time. Having this concept clear, we could say then that there are two possibilities:

1. *Use the RNB injections in order to avoid the functional consequences of a reactivation.* In this case, the injections should be applied at the exact moment that morphological reactivation occurs, which requires the application of sensitive reactivation criteria and strict monitoring.
2. *Use RNB injections to prevent the appearance of recurrences or morphological recurrences and, therefore, their functional consequences*. This criterion is based on individualized treatment regimens and the so-called Treat and Extend (TAE) [27, 28].

The risk rate (λ) of the morphological reactivation calculated in the review can be used to predict the percentage of patients that we should treat according to the Treat and Extend methodology. We can observe that the results obtained by this prediction are very similar to what was observed in the LUCAS study [18] (Fig. 5), which was a comparative study between Bevacizumab and Ranibizumab using said scheme.

In planning the TAE scheme, the existence of a maximum interval of 12 weeks between injections has been established as a criterion. This limit may seem reasonable due to the lack of effectiveness demonstrated by the PIER study (load up + injections at quarterly intervals) [29]; however, there is no evidence to show that it does not make sense to prolong the visits even more. If we consider that in the framework of the clinical trials that used the PRN scheme, there are patients who have received a single maintenance dose of RNB after 12 months of follow-up. It is logical to think then that the reactivation time can be extended beyond 3 months.

Finally, when comparing the reactivation times calculated between RNB and BVZ, we could observe the existence of significant differences between both drugs. Although for a long time, it was thought that Bevacizumab could be applied with a more spaced frequency (every 6 weeks), these results would indicate the opposite. A possible explanation could be found in the affinities for VEGF. The RNB was designed to have greater affinity for the growth factor, something that is reflected in the dissociation constants of both drugs: Kd RNB = 19.8 pM; Kd BVZ = 433 pM [30]. With a similar vitreous half-life, the greater affinity for the molecule would lead to a more lasting effect for RNB. Therefore, the use of RNB enables patients to receive less injections than BVZ throughout the treatment.

In conclusion, reactivation time is a poorly studied variable, but of great importance for the planning of therapeutic schemes for anti-VEGF treatment of AMD. The calculation of reactivation time from the PRN dose number turns out to be a good approximation for the retrospective study of the variable when reactivation times have not been studied directly. In spite of this, we believe that the detailed study of this variable should be incorporated in each of the clinical studies that address this pathology. Exploring the intrinsic and extrinsic factors associated with this variable could provide information on how to reduce the burden on patients without incorporating new drugs to treatment.

Finally, and in relation to the use of the PRN scheme, the results of the comparison between the observational studies and RCTs allow us to conclude that the use of criteria not based on OCT scans delays the application of doses between 2 or 3 weeks, time enough so that the clinical symptoms are expressed and the patient suffers loss of clinical benefits.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval For this type of study, formal consent is not required

References

1. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP, Mariotti SP (2004) Global data on visual impairment in the year 2002. Bull World Health Organ 82(11):844–851
2. Gohdes DM, Balamurugan A, Larsen BA, Maylahn C (2005) Age-related eye diseases: an emerging challenge for public health professionals. Prev Chronic Dis 2(3):A17
3. Wong TY, Chakravarthy U, Klein R, Mitchell P, Zlateva G, Buggage R, Fahrbach K, Probst C, Sledge I (2008) The natural history and prognosis of neovascular age-related macular degeneration: a systematic review of the literature and meta-analysis. Ophthalmology 115(1):116–126
4. MARINA Study Group, Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, Kim RY (2006) Ranibizumab for neovascular age-related macular degeneration. N Engl J Med 355(14):1419–1431
5. ANCHOR Study Group; Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Lanchulev T (2006) Ranibizumab versus Verteporfin photodynamic therapy for neovascular age-related macular degeneration: two-year results of the ANCHOR study. Ophthalmology 116(1):57–65
6. Smiddy WE (2009) Economic implications of current age-related macular degeneration treatments. Ophthalmology 116(3):481–487
7. Real JP, Tártara I, Allemandi D, Granero G, Palma SD (2011) Tratamiento de la degeneración macular asociada a la edad (DMAE). Atención Farmacéutica. (European Journal of Clinical Pharmacy) Barcelona: rasgo editorial. 13(3):160–171
8. Stewart MW (2015) Individualized treatment of neovascular age-related macular degeneration: what are patients gaining? Or losing? J Clin Med 4:1079–1101
9. Monés J, Biarnés M, Trindade F, Casaroli-Marano R (2012) FUSION regimen: ranibizumab in treatment-naïve patients with

exudative age-related macular degeneration and relatively good baseline visual acuity. Graefes Arch Clin Exp Ophthalmol 250(12):1737–1744
10. Zhang Y, Yao Z, Kaila N, Kuebler P, Visich J, Maia M, Tuomi L, Ehrlich JS, Rubio RG, Campochiaro PA (2014) Pharmacokinetics of ranibizumab after intravitreal administration in patients with retinal vein occlusion or diabetic macular edema. Ophthalmology 21(11):2237–2246
11. Holz FG, Amoaku W, Donate J, Guymer RH, Kellner U, Schlingemann RO, Weichselberger A, Staurenghi G (2011) Safety and efficacy of a flexible dosing regimen of ranibizumab in neovascular age-related macular degeneration: the SUSTAIN study. Ophthalmology 118(4):663–671
12. Rebasa P (2005) Basic concepts in survival analysis. Cirugía Española 78(4):222–230
13. Moraleda A, Villalba CM (2013) Modelado y Simulación de Eventos Discretos (ebook), 1ra edicion edn. Uned. Universidad Nacional de Educacion a Distancia, España
14. ARRIBALZAGA EB (2007) Interpretación de las curvas de supervivencia. Rev Chil Cir 59(1):75–83. https://doi.org/10.4067/S0718-40262007000100013
15. Cox C, Chu H, Schneider MF, Muñoz A (2007) Parametric survival analysis and taxonomy of hazard functions for the generalized gamma distribution. Stat Med 26(23):4352–4374
16. Arroyo I, Bravo LC, Llinas H, Munoz FL (2014) Distribuciones Poisson y Gamma: Una Discreta y Continua Relación. Prospect. [online], 12(1):99–107. http://www.scielo.org.co/pdf/prosp/v12n1/v12n1a12.pdf
17. Higgins J, Green S (2011) Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration; http://handbook-5-1.cochrane.org. Accessed 6 Jul 2015
18. Berg K, Hadzalic E, Gjertsen I, Forsaa V, Berger LH, Kinge B, Henschien H, Fossen K, Markovic S, Pedersen TR, Sandvik L, Bragadóttir R (2015) Ranibizumab or Bevacizumab for neovascular age-related macular degeneration according to the Lucentis compared to Avastin study treat-and-extend protocol: two-year results. Ophthalmology 123(1):51–59
19. IVAN study investigators [IVAN], Chakravarthy U, Harding SP, Rogers CA, Downes SM, Lotery AJ, Culliford LA, Reeves BC (2013) Alternative treatments to inhibit VEGF in age-related choroidal neovascularisation: 2-year findings of the IVAN randomised controlled trial. Lancet 382(9900):1258–1267
20. Hörster R, Ristau T, Sadda SR, Liakopoulos S (2011) Individual recurrence intervals after anti-VEGF therapy for age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 249(5):645–652
21. Mantel I, Deli A, Iglesias K, Ambresin A (2013) Prospective study evaluating the predictability of need for retreatment with intravitreal ranibizumab for age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 251(3):697–704
22. Muether PS, Hermann MM, Viebahn U, Kirchhof B, Fauser S (2012) Vascular endothelial growth factor in patients with exudative age-related macular degeneration treated with ranibizumab. Ophthalmology 10:2082–2086
23. Cohen SY, Dubois L, Tadayoni R et al (2009) Results of one-year's treatment with ranibizumab for exudative age-related macular degeneration in a clinical setting. Am J Ophthalmol 148:409–413
24. Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ (2011) Ranibizumab and bevacizumab for neovascular age-related macular degeneration. N Engl J Med 364(20):1897–1908
25. Real JP, Granero GE, De Santis MO, Juarez CP, Palma SD, Kelly SP, Luna JD (2015) Rate of vision loss in neovascular age-related macular degeneration explored. Graefes Arch Clin Exp Ophthalmol 253(11):1859–1865
26. Barquet A, Monés J (2012) New treatment protocols and follow-up in patients with exudative age-related macular degeneration. Arch Soc Esp Oftalmol 87(Suppl 1):10–17
27. Gupta OP, Shienbaum G, Patel AH, Fecarotta C, Kaiser RS, Regillo CD (2010) A treat and extend regimen using ranibizumab for neovascular age-related macular degeneration clinical and economic impact. Ophthalmology 17(11):2134–2140
28. Oubraham H, Cohen SY, Samimi S, Marotte D, Bouzaher I, Bonicel P, Fajnkuchen F, Tadayoni R (2011) Inject and extend dosing versus dosing as needed: a comparative retrospective study of Ranibizumab in exudative age-related macular degeneration. Retina 3:26–30
29. Abraham P, Yue H, Wilson L (2010) Randomized, double-masked, sham-controlled trial of Ranibizumab for neovascular age-related macular degeneration: PIER study year 2. Am J Ophthalmol 150(3):315–324
30. Magdelaine-Beuzelin C, Pinault C, Paintaud G, Watier H (2010) Therapeutic antibodies in ophthalmology: old is new again. MAbs 2(2):176–180