Full title:

Retrospective cohort study exploring whether an association exists between spatial distribution of cystoid spaces in cystoid macular oedema secondary to Retinitis Pigmentosa and response to treatment with carbonic anhydrase inhibitors.

Synopsis:

This retrospective cohort study demonstrates a possible association between the spatial distribution of cystoid spaces in Retinitis Pigmentosa-associated cystoid macular oedema and response to carbonic anhydrase inhibitors.

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Competing interests statement

No, there are no competing interests.

Keywords (MeSH terms):

Carbonic Anhydrase Inhibitor, Cystoid Macular Oedema, Imaging, Macular Oedema, Optical Coherence Tomography, Retinal Diseases, Retinitis Pigmentosa

Structured abstract

Background: Carbonic anhydrase inhibitors (CAIs) are frequently used as an initial step to treat Retinitis Pigmentosa-associated cystoid macular oedema (RP-CMO). Interestingly, it has been postulated that CAIs might reduce outer nuclear layer (ONL) fluid more effectively than inner nuclear layer (INL) fluid due to better access to retinal pigment epithelium basolateral membrane than neurosensory retina. This retrospective cohort study explores if an association between spatial distribution of cystoid spaces in RP-CMO and CAI response exists.

Methods: Two independent graders reviewed pre- and post-treatment OCT images of 25 patients (43 eyes) initiated on topical and/or oral CAI's between January 2013 and December 2014. Documentation included the presence/absence of: fluid (and layer(s) involved), external limiting membrane, epiretinal membrane (ERM), vitreomacular adhesion/traction, lamellar/full-thickness macular hole and central macular thickness (CMT)/volume.

Results: INL fluid was found in all study eyes. All 13 'responders' (at least 11% reduction of CMT after treatment) demonstrated pre-treatment ONL fluid. In 7 patients (4 responders and 3 non-responders) complete clearance of ONL fluid was achieved despite persistence of INL fluid. ERM presence was similar in responders and non-responders.

Conclusion: In this study, INL fluid was found to be the most common spatial distribution of RP-CMO. However, patients who were classed as a 'responder' to CAI treatment, all demonstrated co-existing ONL fluid on their pre-treatment OCT scan. This may be explained by CAIs having better access to retinal pigment epithelium basolateral membrane than neurosensory retina. Our study also suggests a minimal impact on response to CAIs by epiretinal membrane.

Introduction

Inherited retinal disease (IRD) is the leading cause of blindness certification in the working age population (age 16-64 years) in England and Wales [1]. Retinitis Pigmentosa (RP) is the most common group of IRD, causing progressive centripetal reduction of vision and associated with variants in over 80 genes to date, accounting for approximately 50-60% of cases, with an autosomal recessive, autosomal dominant or X-linked mode of inheritance [2]. Secondary complications associated with RP include cataracts and RP-associated cystoid macular oedema (RP-CMO), which further contribute to reduction of visual acuity [3]. RP-CMO has been reported to occur in 10 - 50% of patients [4-7].

Proposed mechanisms for RP-CMO include: 1) breakdown of the blood-retinal barrier (BRB), 2) failure (or dysfunction) of the pumping mechanism in the retinal pigment epithelium (RPE), 3) Müller cell oedema and dysfunction, 4) anti-retinal antibodies and 5) vitreous traction [8].

Makiyama et al [9] used spectral domain optical coherence tomography (SD-OCT) to investigate the prevalence and spatial distribution of cystoid spaces (CS) in RP. Seventy-four of 275 patients (27%) demonstrated RP-CMO in at least one eye. Inner nuclear layer (INL) CS were observed in 99% of eyes with CMO. It is of note that Müller cell bodies reside in the INL, providing support for the aforementioned Müller cell oedema /dysfunction hypothesis. The outer nuclear layer (ONL)/outer plexiform layer was involved in 28% and ganglion cell layer involved in 7%. Interestingly, 79% of CS were located in areas of relatively well-preserved outer retina in keeping with the observation that CMO is seen more commonly in less advanced RP compared to late stage RP [9].

Several case-reports and small-scale studies have been published regarding the safety and efficacy of carbonic anhydrase inhibitors (CAIs) in the treatment of RP-CMO, however, to-date there is currently no level 1 evidence to support this [8]. In the largest retrospective study to date of CAIs involving 81 patients (157 eyes) with RP-CMO, objective improvement on OCT was observed in 53% of patients

(40% of eyes) using topical dorzolamide versus 41% of patients (28% of eyes) using oral acetazolamide [10]. CAIs are associated with a variety of potential side effects, including: fatigue, loss of appetite, limb paraesthesia, kidney stones, aplastic anaemia, hypokalaemia and cardiac arrhythmia [11]. Their mechanism of action is through inhibition of the enzyme carbonic anhydrase IV (CA IV), resulting in acidification of the sub-retinal space, increased chloride ion transport, and subsequent movement of water across the RPE into the choroid [12].

Acetazolamide cannot readily enter the neurosensory retina, yet has good access to the RPE basolateral membrane [13]. It has been previously suggested that CMO with RPE pathology may respond better to CAIs than CMO with retinal capillary pathology[13-15]

We have therefore undertaken a retrospective cohort study designed to determine if there is an association between the spatial distribution of CS in RP-CMO and response to CAIs.

Materials and methods

This retrospective cohort study identified all patients seen at Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom between January 2013 and December 2014 with 'Retinitis Pigmentosa' and 'Cystoid Macular Oedema' appearing in their electronic patient record. This time period was chosen as it was deemed a manageable period of time and number of scans to analyse. This initial search identified 103 patients, however, after review of each patient record, 78 patients were excluded from the study due to having 'no' cystoid macular oedema. This study was IRB approved.

Patients were subsequently included in the study if they met the following inclusion criteria (irrespective of age): (i) Confirmed diagnosis of RP-CMO; (ii) Unilateral or bilateral (if bilateral, each eye was evaluated individually) RP-CMO; (iii) Commenced on treatment with either a topical and/or

oral CAI between January 2013 and December 2014; (iv) Pre-treatment SD-OCT scan acquired within 2 weeks of initiating treatment AND post-treatment SD-OCT scan acquired between 3 – 9 months after initiation of treatment. This time period was chosen because whilst patients in our clinics are typically reviewed at 3 – 4 months after treatment initiation, their appointments may be postponed due to various factors.

Patients were excluded if any of the following applied: (i) A diagnosis of CMO not considered to be related to RP; (ii) Other treatment for RP-CMO received within 3 months of initiation of CAI e.g. intravitreal injection of triamcinolone/anti-VEGF agent; (iii) Pre-treatment SD-OCT scan not acquired within 2 weeks of initiating treatment and/or post-treatment OCT scan acquired greater than 9 months from initiation of treatment.

After accounting for the inclusion and exclusion criteria, the total number of patients included in the study totalled 25. Of these, 18 had bilateral RP-CMO and 7 had unilateral RP-CMO; 43 eyes graded in total.

SD-OCT (Spectralis, Heidelberg Engineering Ltd, Heidelberg, Germany) was undertaken in all recruited subjects. All patients across the cohort underwent scan acquisition according to the standard of care procedure used for out-patient clinics at Moorfields Eye Hospital, London.

Image Grading

Two independent graders experienced in SD-OCT interpretation graded pre-and post-treatment scans (SS, NH). Both graders were blinded to the treatment that each patient received and whether they were classed as a 'responder' or not.

Each grader began by performing re-centration of the images if deemed necessary. The following variables were graded for their presence within 3600 microns of the foveal centre: sub-retinal fluid (SRF), inner nuclear layer (INL) fluid, outer nuclear layer (ONL) fluid, ganglion cell layer fluid, epi-retinal membrane (ERM), vitreo-macular adhesion (VMA), vitreo-macular traction (VMT), lamellar macular hole and full-thickness macular hole (FTMH). Each of these variables was graded as either: present (>90% certainty), questionably present (50-90% certainty), absent (<50% certainty) or ungradable. The presence of external limiting membrane (ELM) within 1200 microns of the foveal centre was also graded and a comment made whether it was felt to be intact throughout or disrupted. A further 'yes' or 'no' response was required for the grading of whether there was felt to be intact ELM and/or fluid present (in any lamination) directly under the foveal centre. Pre- and post- treatment CMT and macular volume were also documented.

If both graders agreed on a variable, the grading was complete. If the second grader (NH) disagreed with the first grader (SS), adjudication was performed by a consultant retinal specialist (MM). CMT values were considered to be in agreement if graded within 50 microns of each other. Macular volume values were considered to be in agreement if graded within 1.5mm^3 of each other. The mean of the grader scores for CMT was used for analysis. Patients were considered to have responded to treatment if they achieved a CMT reduction of 11.0% or greater in keeping with previous studies [16,17]. Although these studies were published over 8 years ago, no studies have since been published providing an alternative percentage reduction of CMT. We therefore chose this figure to allow comparison with other studies.

Pre- and post-treatment visual acuity was also documented.

Statistical Analysis

Descriptive statistics have been used to describe the results of the CARAMEL study. For categorical variables, Kappa statistic was computed with respective 95% confidence interval (CI) for assessing inter-rater agreement as Kappa is thought to be a more robust measure than simple percent agreement (Kappa takes into account the possibility of agreement occurring by chance). For continuous variables Bland-Altman agreement methods were used to quantify limits of agreement (LoA). Analysis was performed in STATA version 13 (STATA Corp., Texas, USA) and 95% CIs for Kappa were computed using the kapci package using 1000 bootstrap replicates.

Results

Forty three eyes (22 right; 21 left); were included in the study consisting of 18 patients with bilateral RP-CMO and 7 patients with unilateral RP-CMO. Seventeen of these patients were male and 8 were female. Median age was 48 and ranged between 17 and 79 years. Four out of 43 (9.3%) eyes were treated with oral acetazolamide 250mg twice a day versus 39 out of 43 (90.7%) of eyes treated with topical dorzolamide or brinzolamide three times a day. All 43 eyes in the study were graded as having INL fluid present on their pre-treatment OCT scan. Thirty three out of 43 eyes (76.7%) in the study demonstrated co-existing ONL fluid present on their pre-treatment OCT scan. Eleven out of 43 eyes (25.6%) in the study demonstrated co-existing RGC layer fluid present on their pre-treatment OCT scan. No patients demonstrated the presence of sub-retinal fluid.

Estimates of agreement for all variables assessed by the two graders are presented in Table 1 (please see supplementary table 1), Figure 1 and Figure 2. No single variable was found to have poor agreement. No evidence of bias was found in terms of inter-rater agreement for pre-treatment CMT, mean difference -0.74. 95% CI (-3.12, 1.63). Inter-rater LoA were -16.18 to 14.69 for pre-treatment CMT and -18.36 to 21.76 for post-treatment CMT, which was considered by the chief investigator to

be acceptable. Inter-rater agreement was -0.91 to 1.14 for pre-macular volume and -1.22 to 1.24 for post-macular volume, which was considered by the chief investigator to be acceptable with no evidence of bias.

[Insert Figure 1]

[Insert Figure 2]

Out of 43 eyes that were graded, 13 (30.2%) were classed as 'responders' having achieved a CMT reduction following treatment of at least 11%. All 13 responders demonstrated ONL fluid on their pre-treatment OCT and the presence of fluid (in any layer) directly under the fovea. ERM was 'definitely present' in 8 out of 13 (61.5%) responders and 'questionably present' in 2 out of 13 (15.4%) responders. No responder demonstrated VMA, VMT or a FTMH on their pre-treatment OCT scan. ELM was considered to be present (intact or disrupted) within 1200 microns of the fovea and present directly under the fovea in all but 1 responder (92.3%). Best corrected visual acuity (BCVA) improved by at least 10 ETDRS letters in 2 out of 13 (15.4%) responders. No responders demonstrated a loss of 10 or more ETDRS letters. The remaining 11 out of 13 (84.6%) responders demonstrated no change in their BCVA following treatment. Interestingly, 4 out of 13 (30.8%) responders demonstrated total clearance of ONL fluid on their post-treatment OCT scan whilst INL cysts remained

Out of 30 non-responders, 20 (66.7%) eyes had ONL fluid on their pre-treatment OCT compared to 10 (33.3%) eyes without ONL fluid on their pre-treatment OCT. Sixteen out of 30 (53.3%) non-responder eyes had fluid (in any layer) directly under the fovea. Interestingly, there were three non-responders that demonstrated total clearance of ONL fluid on their post-treatment OCT scan whilst INL cysts remained (see Figure 3). VMA was definitely present in 7 out of 30 (23.3%) non-responders

and questionably present in 2 out of 30 (6.7%) non-responders. ELM was considered to be present (intact or disrupted) within 1200 microns of the fovea in 26 out of 30 (86.7%) non-responder eyes, however, was only present directly under the fovea in 20 out of 30 (67.0%) eyes. BCVA improved by at least 10 ETDRS letters in 2 out of 30 (6.7%) non-responders. No non-responders demonstrated a loss of 10 or more ETDRS letters. The remaining 28 out of 30 (93.3%) non-responders demonstrated no change in their BCVA following treatment.

Of note, all 4 patients (2 responders and 2 non-responders) who gained at least 10 ETDRS letters of BCVA, demonstrated ONL fluid on their pre-treatment OCT scans.

Discussion

In keeping with Makiyama et al [9], this study observed an overall higher frequency of INL compared to ONL fluid in patients with RP-CMO [9]. It was interesting to note that 100% of responders demonstrated ONL fluid on their pre-treatment OCT; however, not every patient with pre-treatment ONL fluid responded. The presence of ERM graded as either 'questionably present' or 'definitely present' was similar in both groups (10 out of 13 (77.0%) responder eyes versus 24 out of 30 (80.0%) non-responder eyes) and therefore does not appear to significantly affect response to treatment in our cohort. In contrast, the presence of VMA graded as either 'questionably present' or 'definitely present' was greater in the non-responder group (0 out of 13 (0.0%) responder eyes versus 9 out of 30 (30.0%) non-responder eyes) and may therefore play a role in limiting response to treatment as being indicative of a tractional component that would not be expected to respond to CAIs.

Overall, there were seven patients (including 'responders' and 'non-responders') that demonstrated total clearance of ONL fluid on their post-treatment OCT scan despite persistence of INL cysts. Our working hypothesis to explain this response includes the closer anatomical proximity of ONL fluid to the RPE where the action of CAI's take place. A greater percentage of eyes in the responder group

demonstrated at least 10 ETDRS letter improvement of BCVA compared to the non-responder group (15.4% of responders versus 6.7% of non-responders). It is not uncommon for anatomical improvement to occur without significant functional improvement[18]. Factors such as underlying photoreceptor loss and/or chronicity of RP-CMO may have an effect on visual outcome[8,18]. Whilst it was noted that patients with visual gain demonstrated ONL fluid on their pre-treatment OCT scans, a larger study with appropriate statistical analysis is required to be able to qualify whether this observation is significant.

In 2015, Liew et al [10] conducted the largest retrospective study to date involving 81 patients (157 eyes) with RP-CMO. A positive response to treatment was only observed in 53% of patients (40% of eyes) using topical dorzolamide and 41% of patients (28% of eyes) using oral acetazolamide [10]. CAIs are also associated with side-effects such as: tingling/numbness of the limbs, fatigue, renal stones, aplastic anaemia, hypokalaemia and cardiac arrhythmia [11]. To this end, it would be valuable to identify factors that might help predict response of RP-CMO to CAI treatment in order to tailor patient care appropriately. Whilst this study might suggest the presence of ONL fluid on pre-treatment OCT scan to be a positive prognostic factor in the treatment of RP-CMO, we are limited by design and numbers to be able to provide accurate statistical analysis and it must therefore be interpreted as observation only

There is currently no level 1 evidence for the treatment of RP-CMO. The following recommendations are therefore based on expert opinion:

1) Initial treatment using a topical CAI and review after 4-6 months.
2) a) After 4-6 months, if there has been minimal (but not significant) reduction of CMT or improvement of BCVA, options may include switching to a different topical CAI agent or oral CAI.

b) After 4-6 months, if there has been no reduction of CMT or improvement of BCVA or if the spatial distribution of cysts are located only in the INL, consideration should be given to trialling alternative therapeutic options, such as: topical non-steroidal anti-inflammatory agents or considering intravitreal anti-VEGF (although these latter agents are currently unlicensed for RP-CMO).

Although there was only a limited vision-improving effect observed in this study, we still believe in treating RP-CMO to achieve anatomical improvement to prevent irreversible structural damage as well as to potentially decelerate underlying photoreceptor loss[8].

Studies currently being undertaken for the treatment of RP-CMO include: The AMOUR study to assess the safety and efficacy of aflibercept for RP-CMO (clinicaltrials.gov identifier NCT02661711).

Our study has several limitations including the small cohort size, with further studies with larger patient numbers required in order to support our findings of OCT phenotypes that may help predict treatment response in patients with RP and thereby better inform patient counselling and management in this highly genetically heterogenous retinal dystrophy.

Contributorship statement

S A Strong:

- Substantial contributions to the design of the work; the acquisition, analysis, and interpretation of data for the work
- Drafting the work
- Final approval of the version to be published
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

N Hirji:

- Substantial contributions to the design of the work; the acquisition, analysis, and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

A Quartilho:

- Substantial contributions to the design of the work; analysis, and interpretation of data for the work
- Drafting the work
- Final approval of the version to be published
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

A Kalitzeos:

- Final approval of the version to be published
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

M Michaelides:

- Substantial contributions to the conception and design of the work; the acquisition, analysis, and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Table 1. Inter-rater agreement for pre and post-treatment variables assessed by the two graders

Figure 1. Bland-Altman graph for pre CMT inter-rater agreement

Figure 2. Box plot illustrative of pre and post CMT measurements made by the two graders

Figure 3. Two examples where ONL cysts have disappeared following treatment with CAIs, yet INL cysts remain. 1a and 2a = Pre-treatment SD-OCT scans of two separate patients who were classed as a 'non-responder' in the CARAMEL study (the white arrows demonstrate the presence of ONL fluid cysts). 1b and 2b = Post-treatment SD-OCT scans with absence of ONL fluid cysts yet INL cysts remain.

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