# Proposal

## The Institutional Review Board of the Faculty of Medicine Vajira Hospital

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**1. Study title**

Effects of antiplatelet and antioxidant agents on drusen progression: A pilot, prospective cohort study

**2. Principal Investigator**


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**3. Co-investigator**

None

**4. Background and Rationale**

Age-related macular degeneration (AMD) is one of the leading causes of blindness in the elderly. The incidence of AMD is 8.69% worldwide (1) and 6.8%–7.38% in Asia (1, 2). AMD is classified as dry AMD/non-neovascular AMD or wet AMD/neovascular AMD.

Drusen, a small yellowish spot, is a hallmark of AMD. Early stages of AMD will present only drusen and asymptomatic, progressing to retinal pigmented epithelium (RPE) abnormalities later, and progressing to geographic atrophy in late/advanced or advanced stages of AMD (3, 4).

AMD is caused by a variety of mechanisms, including genetic factors, waste product accumulation in the RPE, which causes mitochondrial function abnormalities, resulting in RPE destruction and cell death. Furthermore, reactive oxygen species in the retina caused by inflammation and cell death promote RPE dysfunction and death as a vitreous cycle (3-5). Also, from choriocapillaris vascular ischemia (6).

The progression of disease from the early stages to the advanced stages is 5–6.6 years (7, 8) and causes irreversible visual loss. In age-related eye disease studies (AREDS), antioxidant supplements consist of vitamin C, vitamin E, zinc, copper, lutein, and zeaxanthin. According to the study, supplements can reduce the risk of progression to advanced AMD by 25% in Category 3 and 4 (9, 10). Because there is no specific treatment for AMD other than slowing the rate of progression, several studies are required to discover AMD treatment.

Antiplatelet drugs can reduce rate of progression to geographic atrophy by reducing choriocapillaris ischemia (11) and do not increase the rate of bleeding when compared to no medication (11, 12).

Some studies showed that antioxidants such as N-acetylcysteine, vitamin C, and vitamin E can protect RPE from oxidative stress and decrease cell death by reducing reactive oxygen species (9, 10, 13-15). In addition, drusen decreased and no geographic atrophy was observed in a dry AMD patient who took daily supplements (16). Therefore, antioxidants such as N-acetylcysteine may have benefits in dry AMD with minor side effects (17-19).

The aim of this clinical trial is to evaluate the effect of low doses of antiplatelet medications (aspirin 81 mg/day or clopidogrel 75 mg/day) with or without a combination of antioxidants (N-acetylcysteine 600 mg/day) in a dry AMD patient with large drusen.

## 5. Propose

5.1 Primary Objective

To evaluate dry AMD progression in participants with large drusen by drusen volume analysis.

Participants will divide in to three groups.

- Participants who were already taking low dose antiplatelet medications (aspirin 81 mg/day or clopidogrel 75 mg/day).
- Participants who take the antiplatelet drug mentioned above in addition to the antioxidant (N-acetylcysteine 600 mg/day) prescribed by the investigator.
- Participants does not use any medications.

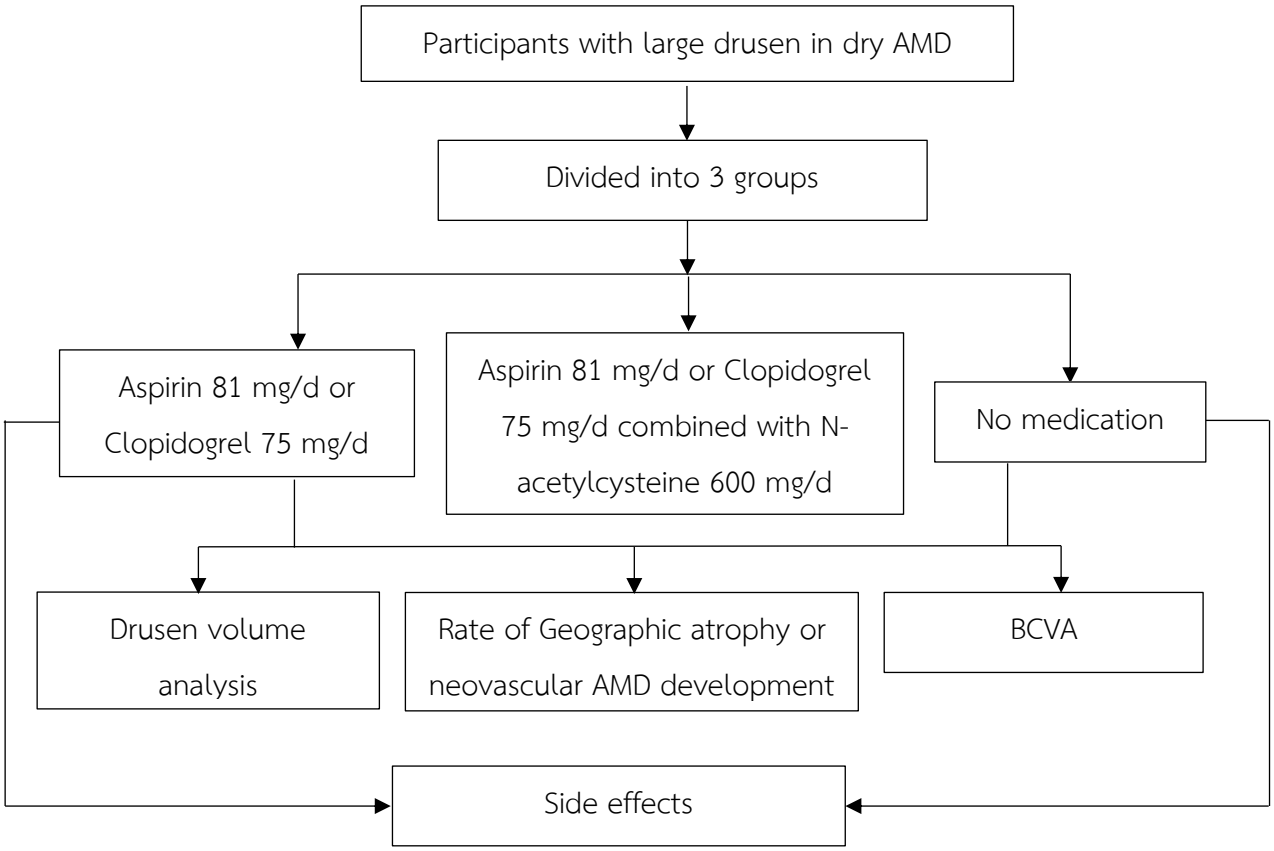
5.2 Secondary Objectives

5.2.1 Best-corrected visual acuity/BCVA of participants by groups in 6.1

5.2.2 Rate of progression to geographic atrophy or neovascular AMD

5.2.3 Side effects and safety

## 6. Framework




## 7. Material and Method

### 7.1 Study Design

Prospective interventional cohort study

### 7.2 Study Population

Participants with dry AMD or non-neovascular AMD who have large will be divided into three groups.

1. Participants who were already taking low dose antiplatelet medications (aspirin 81 mg/day or clopidogrel 75 mg/day).

2. Participants who take the antiplatelet drug mentioned above in addition to the antioxidant (N-acetylcysteine 600 mg/day) prescribed by the investigator.
3. Participants does not use any medications.

Participants must take medication for at least 12 months. Antiplatelet drugs are medications that patients take to treat their underlying condition, such as aspirin 81 mg/day or clopidogrel 75 mg/day. Participants using antiplatelet medications will be randomly assigned to receive N-acetylcysteine or not.

**7.3 Inclusion, exclusion criteria and Criteria for discontinuation**

Inclusion criteria

1. Dry AMD with at least 1 large drusen. According to the AREDS study, large drusen have a size of more than 125 micron.(21)

2. Patients can evaluate SD-OCT (Spectral domain optical coherence tomography), OCT angiography, and best-corrected visual acuity.

3. Age range: 50-85 years

4. Patients who have previously used antiplatelet drugs.

Exclusion criteria

1. Patient with advanced AMD, such as geographic atrophy, neovascular complications (choroidal neovascularization)

2. Patient with additional retinal diseases that affect visual acuity, e.g., retinal detachment, diabetic macular edema.

3. Patient with a history of intravitreal anti-VEGF injection or macular laser.

4. Patient using SSRIs, SNRIs, azole, NSAIDs, dual antiplatelet, anticoagulant medications.

Criteria for discontinuation

1. Participants who cannot tolerate the side effects of N-acetylcysteine. However, the side effects of N-acetylcysteine are mild, such as heartburn, nausea, and vomiting.(17, 22)

2. Participants need to stop antiplatelet drugs prescribed by their doctor.

3. Participants cannot evaluate their BCVA or undergo SD-OCT or OCT angiography.

**7.4 Sample size**

Previous study showed drusen volume accumulation before the advanced stage was 0.264 ± 0.013 $mm^3$ per year (23). The AREDS formula can reduce progression to the advanced AMD by 4% each year. The researchers believe that using antiplatelet medication alone or in combination with antioxidants will decrease progression by 10%, or 0.211 mm3.

$$N = \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 \sigma^2}{(\mu - \mu_0)^2}$$

reference value ($\mu_0$) = 26.4

mean ($\mu$) = 21.1

standard deviation ($\sigma$) = 13

alpha ($\alpha$) = 0.05

beta ($\beta$) = 0.2

Estimated sample size:

N = 48

Sample size = 48 eyes per group. Some participants lost 20% throughout the follow-up period. As a result, the sample size is 174 eyes (58 eyes per group).

**7.5 Variables**

Independent Variable – 3 medications groups 1. Aspirin 81 mg per day or Clopidogrel 75 mg per day 2. Aspirin 81 mg per day or Clopidogrel 75 mg per day combined with N-acetylcysteine 600 mg (NAC-long, Temmler Pharma, Marburg, Germany) per day

Dependent variable - Drusen volume analysis, rate progression to geographic atrophy or neovascular AMD, and BCVA

**7.8 Methodology**

1. Application for ethic review

2. Select dry AMD participants based on inclusion and exclusion criteria. Participants sign an informed consent form.

3. Participants will be divided into three groups.

- Participants who were already taking low dose antiplatelet medications (aspirin 81 mg/day or clopidogrel 75 mg/day).

- Participants who take the antiplatelet drug mentioned above in addition to the antioxidant (N-acetylcysteine 600 mg/day) prescribed by the investigator.

- Participants does not use any medications.

Participants must take medication for at least 12 months. Antiplatelet drugs are medications that patients take to treat their underlying condition, such as aspirin 81 mg/day or clopidogrel 75 mg/day. Participants using antiplatelet medications will be randomly assigned to receive N-acetylcysteine or not.

4. The date the medication receipt is day 1. Participants must follow up every three months for a total of twelve months. Participants will examine BCVA, take a fundus photo, SD-OCT, and OCT angiography, and evaluate side effects from medication and compliance.

**7.9 Statistical analysis**

1. Quantitative data is drusen volume analysis, rate of progression to geographic atrophy or neovascular AMD, using ANOVA test; multiple regression analysis by confounder adjustment, pairwise comparison, and linear mixed modal analysis. A p-value of $< 0.05$ was considered statistically significant.

2. Qualitative Data is side effect from medications.

## 8. Reference


1. Wong WL, Su X, Li X, Cheung CMG, Klein R, Cheng C-Y, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. The Lancet Global Health. 2014;2(2):e106-e16.

2. Kawasaki R, Yasuda M, Song SJ, Chen S-J, Jonas JB, Wang JJ, et al. The prevalence of age-related macular degeneration in Asians: a systematic review and meta-analysis. Ophthalmology. 2010;117(5):921-7.

3. Ambati J, Fowler BJ. Mechanisms of age-related macular degeneration. Neuron. 2012;75(1):26-39.

4. Buschini E, Piras A, Nuzzi R, Vercelli A. Age related macular degeneration and drusen: neuroinflammation in the retina. Progress in neurobiology. 2011;95(1):14-25.

5. Deng Y, Qiao L, Du M, Qu C, Wan L, Li J, et al. Age-related macular degeneration: Epidemiology, genetics, pathophysiology, diagnosis, and targeted therapy. Genes & diseases. 2022;9(1):62-79.
6. Biesemeier A, Taubitz T, Julien S, Yoeruek E, Schraermeyer U. Choriocapillaris breakdown precedes retinal degeneration in age-related macular degeneration. Neurobiology of aging. 2014;35(11):2562-73.
7. Ferris III FL, Wilkinson C, Bird A, Chakravarthy U, Chew E, Csaky K, et al. Clinical classification of age-related macular degeneration. Ophthalmology. 2013;120(4):844-51.
8. Klein ML, Ferris III FL, Armstrong J, Hwang TS, Chew EY, Bressler SB, et al. Retinal precursors and the development of geographic atrophy in age-related macular degeneration. Ophthalmology. 2008;115(6):1026-31.
9. Group A-REDSR. Lutein+ zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. Jama. 2013;309(19):2005-15.
10. Group A-REDSR. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Archives of ophthalmology. 2001;119(10):1417-36.
11. Chantarasorn Y, Smitthimathin W, Vorasayan P. The Role of Dual Antiplatelets in Geographic Atrophy Secondary to Non-Neovascular Aged-Related Macular Degeneration. Frontiers in Ophthalmology.56.
12. Ying G-s, Maguire MG, Daniel E, Grunwald JE, Ahmed O, Martin DF, et al. Association between antiplatelet or anticoagulant drugs and retinal or subretinal hemorrhage in the comparison of age-related macular degeneration treatments trials. Ophthalmology. 2016;123(2):352-60.
13. Terluk MR, Ebeling MC, Fisher CR, Kapphahn RJ, Yuan C, Kartha RV, et al. N-Acetyl-L-cysteine protects human retinal pigment epithelial cells from oxidative damage: implications for age-related macular degeneration. Oxidative medicine and cellular longevity. 2019;2019.
14. Carver KA, Yang D. N-acetylcysteine amide protects against oxidative stress–induced microparticle release from human retinal pigment epithelial cells. Investigative ophthalmology & visual science. 2016;57(2):360-71.

15. Wu J, Cui D, Li H, Zeng J. Protective effects of NAC and salubrinal on apoptosis of retinal pigment epithelial cells induced by all-trans retinoic acid. European Journal of Ophthalmology. 2022;32(1):395-401.
16. Zeng R, Garg I, Miller JB. Complete Resolution of Central Soft Drusen without Geographic Atrophy or Choroidal Neovascularization. Journal of Clinical Medicine. 2022;11(6):1637.
17. Tenório MCdS, Graciliano NG, Moura FA, Oliveira ACMd, Goulart MOF. N-acetylcysteine (NAC): impacts on human health. Antioxidants. 2021;10(6):967.
18. Qi Q, Ailiyaer Y, Liu R, Zhang Y, Li C, Liu M, et al. Effect of N-acetylcysteine on exacerbations of bronchiectasis (BENE): a randomized controlled trial. Respiratory research. 2019;20:1-10.
19. Calverley P, Rogliani P, Papi A. Safety of N-acetylcysteine at high doses in chronic respiratory diseases: a review. Drug safety. 2021;44:273-90.
20. Balaratnasingam C, Yannuzzi LA, Curcio CA, Morgan WH, Querques G, Capuano V, et al. Associations between retinal pigment epithelium and drusen volume changes during the lifecycle of large drusenoid pigment epithelial detachments. Investigative ophthalmology & visual science. 2016;57(13):5479-89.
21. Group A-REDSR. The age-related eye disease study (AREDS): design implications AREDS report no. 1. Controlled clinical trials. 1999;20(6):573.
22. Stey C, Steurer J, Bachmann S, Medici T, Tramer M. The effect of oral N-acetylcysteine in chronic bronchitis: a quantitative systematic review. European Respiratory Journal. 2000;16(2):253-62.
23. Balaratnasingam C, Yannuzzi LA, Curcio CA, Morgan WH, Querques G, Capuano V, et al. Associations Between Retinal Pigment Epithelium and Drusen Volume Changes During the Lifecycle of Large Drusenoid Pigment Epithelial Detachments. Invest Ophthalmol Vis Sci. 2016;57(13):5479-89.

# Informed Consent Form

Date……..

I, ....., Age ..... years old,

Adress ....., Province .....,

City ....., Postal code ....., Phone number .....

Hereby, declare my intention that I consent to be a participant of the research project effects of Antiplatelet and Antioxidant Agents on Drusen Progression: A Pilot, Prospective Cohort Study. I have received the Participant Information Sheet, read through and understood all the details. I have been informed of the details of the research objectives, details of the process steps that the participant must perform, duration of the participation, benefits and risks that will occur of the participation, preventive and corrective measures, compensation to be received, and expenses that I must be responsible for (if any). Also, the principal investigator has already given explanation and completely answered all my queries.

I acknowledge that I have the right to receive additional information on both benefits and harms from participating this research and be able to withdraw or cease my participation any time without any impact on my study in the future. I also consent the researcher to use my personal data for the research and present the research results as an overview only.

Should there be any unusual symptoms, sick feeling and/or impact on my mental occurred during participating in the research, I shall promptly inform the researcher.

Should I have any queries regarding the research procedure or there be any undesirable side effects incurred from participating in the research, I shall be able to contact Dr. Yolradee Winuntamalakul, phone number +6685-1283362.

If I am not treated as stated in the Participant Information Sheet, I shall be able to contact the chairman of the Institutional Review Board of the Faculty of Medicine Vajira Hospital, Navamindradhiraj university, phone number + 662-2443843.

I have thoroughly read and fully understood the contents. Therefore, I give my consent to participate in the research voluntarily and willingly. I have signed this Informed Consent Form.

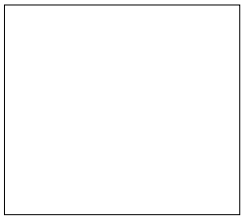
Sign ..... Participant / Date.....

(.....)

Sign..... Information Provider and Consent Requester/Principal Investigator / Date.....

(.....)

In case the participant is incapable of reading or writing, the person who read the entire contents to the participant until understands. I give my consent to participate in the research using fingerprint.



Sign..... Witness / Date.....

(.....)

Name ..... Participant