# The role of CRP and inflammation in the pathogenesis of age-related macular degeneration

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**Abstract**

Age-related macular degeneration (AMD) is a complex, degenerative and progressive disease involving the multiple genetic and environmental factors that can result in severe visual loss. The etiology of AMD is not well understood. Many theories exist and feature mechanisms of oxidative stress, atherosclerotic-like changes, genetic predisposition and inflammation. The most recent clinical studies appointed to a great role of inflammation and C-reactive protein (CRP) in the pathogenesis of AMD. There is a large body of evidence indicating the association of CRP with endothelial dysfunction, oxidative stress and production of reactive oxygen species (ROS), as well as with lipid status disorder in AMD patients. According to recent studies, CRP is definitely not only the inflammatory marker but also a mediator of development of the vascular disorders in the retinal circulation. The results obtained from the present studies may help our understanding the pathogenesis of the retinal vascular disease associated with high levels of CRP.

**Key words**: age-related macular degeneration; C-reactive protein; inflammation

*Received: July 28, 2011* *Accepted: November 30, 2011*

## Introduction

Age-related macular degeneration (AMD) is the most common cause of visual impairment in the individuals over 50 years of age, with the prevalence of 0.05% before the age of 50 rising to 30% after 74 years of age. It is a complex, degenerative and progressive disease involving the multiple genetic and environmental factors that can result in severe visual loss. The molecular mechanisms causing the AMD remain unknown, although inflammatory processes have been implicated by identification of the AMD susceptibility genes encoding complement factors (1) and the presence of the complement proteins in drusen (2).

One of the pathological hallmarks of AMD is the focal deposition of the extracellular material between the retinal pigmented epithelium (RPE) and Bruch's membrane called drusen. Drusen are visualised as yellow deposits under the retinal pigment epithelium and neurosensory retina and are associated with atrophy and depigmentation of the overlying retinal pigment epithelium. Although a few small (< 65 μm) hard drusen can be found in at least 96% of aged population, the presence of a numerous larger (> 125 μm) hard drusen, and especially large, soft drusen (125-250 μm) in the macula is considered particularly when accompanied by pigment irregularities or depigmentation a major risk factor for developing the advanced form of AMD (3). The material, referred to as drusen is composed of several cellular and humoural constituents of systemic inflammatory and immune mediated processes such as HLA-DR, immunoglobulin

λ and κ light chains, complement components 5 and 9, amyloid A, amyloid P component, fibrinogen, vitronectin and C-reactive protein (4). Moreover, the accumulation of drusen can damage the surrounding structures, inculding Bruch's membrane, and is associated with visual deficit that precede the loss of visual accuity in AMD (5).

The aetiology of AMD is not well understood. Many theories exist and feature mechanisms of oxidative stress, atherosclerotic-like changes, genetic predisposition and inflammation (6).

## Risk factors for AMD

Several risk factors have been postulated to take an important role in development of AMD. Age is the strongest risk factor for AMD. The prevalence of AMD increases with age in white individuals (7). Female gender may be a risk factor in individuals aged over 75 years with the relative risk for neovascular form of AMD as much as twice that observed in age-matched men (8).

AMD is more common in white individuals than in people of other ethnic origin (9). It is postulated that increased levels of melanin could increase the free-radical scavenging potential of the RPE and Bruch's membrane, thereby protecting against the risk of AMD (10). Several studies have found an association between advanced AMD and complement factor H, an integral component of the alternative pathway of complement activation (11). Other factors such as factor B and complement components C2 and C3 are also associated with AMD (12). A few clinical trials showed a relationship between the development of exudative lesions and a history of current cigarette smoking. Smoking increases the risk of the exudative type of AMD 2.8 times for females and 3.2 times for current smokers in men. Smoking cessation lowers the relative risk of AMD (13). Some studies have shown a direct association between age-related macular degeneration and raised concentration of cholesterol both in the serum (14) and in the diet (15). Increased concentration of HDL-cholesterol is considered to be cardioprotective have been shown to be associated with a reduced risk of AMD (16).

Several studies have described the beneficial effects of dietary carotenoids in slowing the course of the disease. A multicentre randomized trial has shown that oral supplementation with high levels of antioxidants and minerals are effective in slowing the progression of advanced stages of AMD (17). Some case-control studies have found evidence of decreasing risk of neovascular AMD among individuals reporting the highest intake of omega-3 fatty acids and fish (18). The use of exogenous supplements of oestrogen in post-menopausal women was associated with a lower risk of AMD in a study performed by the Eye Case Control Study Group (17).

It has been postulated that light plays a role in the development of AMD. It has been hypothesized that the photosensitization reactions may be involved in the development of AMD, via synthesis of the reactive oxygen species such as: superoxide, hydrogen peroxide, and singlet oxygen, which may damage the RPE and Bruch's membrane (19). Blue iris color has been inconsistently implicated as a risk factor for AMD (20). Results from Beaver Dam Study suggest that people who spent leisure time outdoors were at increased risk of developing early AMD (21).

Chronic conditions and diseases such as atherosclerosis (22), diabetes (23) and cardiovascular diseases (24) as well are known as risk factors for AMD.

## The pathological features of AMD

The pathology of age-related macular degeneration is characterized by degenerative changes involving the outer portion of the retina, retinal pigment epithelium, Bruch's membrane, and less prominently the choriocapillaris. AMD may be classified into three forms: early, intermediate, and advanced. The early and intermediate forms account for 90% of all cases. In contrast, the advanced form accounts for 88% of all cases of blindness attributable to AMD. The earliest signs of AMD are discrete yellow deposits in the deep layers of the macula, known as drusen. Furthermore, areas of pigmentary disturbance may be observed in the underly-

ing retinal pigment epithelium of the macula. Visual loss associated with these changes may be gradual, resulting from atrophy of the retinal pigment epithelium and the overlying photoreceptors. In addition, the early form of AMD may progress to the intermediate and advanced forms.

Although the advanced form of AMD is less common than the early and intermediate forms, the potential visual loss with advanced AMD is more significant. Advanced AMD has two clinical subtypes. Wet AMD (exudative or neovascular AMD) is the more common subtype and is characterized by proliferation of abnormal vessels in the choroid (a highly vascular area between the sclera and the retinal pigment epithelium). These choroidal neovascular membranes may proliferate into the subretinal space and retina and leak fluid and blood, causing damage and loss of vision. Other features of wet AMD are detachment of the retinal pigment epithelium and fibrosis, often termed a disciform scar, which forms in the late stages of the disease (25).

Pathologic states such as hypoxia, ischemia, or inflammation may tip the balance of proangiogenic and antiangiogenic factors in favor of the formation of new blood vessels. Vascular endothelial growth factor (VEGF) is pivotal in ocular angiogenesis because it is highly selective for endothelial cells, hypoxia drives its synthesis, it diffuses to its target, and it affects multiple components of angiogenesis such as endothelial cell proliferation, survival, and migration. Basic and clinical research implicates VEGF in the pathogenesis of choroidal neovascularization (CNV). Therefore, intravitreal drugs that block VEG have revolutionized the care of patients with neovascular AMD, decreasing growth and leakage from choroidal neovascular lesions and preventing moderate and severe vision loss associated with this process (26,27).

The second subtype of advanced AMD (the dry AMD form) involves atrophy, which progresses to visually significant structures of the retina, such as the fovea, which is responsible for the sharpest and central visual acuity. Progressive atrophy over a large area is termed geographic atrophy and may result in severe visual loss. Geographic atrophy is seen as normal RPE with hypotrophy, hypertrophy hypo- or hyperpigmentation, atrophy, migration, loss of outer retinal cells, attenuation of Bruch's membrane and choriocapillaris degeneration (28).

## The role of inflammation in the pathogenesis of AMD

There is mounting evidence from laboratory based studies that inflammation plays a key role in the pathogenesis of AMD (4,6,29). The inflammatory marker CRP has recently been shown to be an independent risk for cardiovascular and peripheral arterial disease (30-32) and a pathogenic factor leading to endothelial dysfunction in the cell culture model (33). Moreover, elevated concentration of CRP has been associated with an increased risk for hypertension (34), and for type 1 and type 2 diabetes mellitus (35). Because hypertension and diabetes are considered major risk factors for retinal vascular disorders, their association with inflammation and endothelial dysfunction has been suggested in humans with retinopathy (36).

Atherosclerosis is a known risk factor for AMD, most likely through decreased choroidal blood flow, directly or indirectly impairing the functioning of the RPE (37,38). Atherosclerosis is also associated with elevated hsCRP concentration, which may contribute to the higher risk of AMD (39).

Local inflammatory and immune-mediated events play a role in the development of drusen (40-42). Direct analysis by liquid chromatography and immunocytochemical analyses confirmed that drusen contain proteins associated with inflammation such as fibrinogen, vitronectin, complement components and C-reactive protein (CRP) (43). Some of these proteins seem to be locally produced by damaged retinal pigment epithelium cells (42). Drusen components have been found in atherosclerotic plaques and deposits in Alzheimer disease (44), and AMD, atherosclerosis and Alzheimer disease may partly share a similar inflammatory pathogenesis.

The AMD lesion formation has been conceptualized as sharing mechanisms with atherosclerotic

plaque formation, where LDL retention within the arterial wall initiates a cascade of pathologic events called the "response to retention hypothesis" (45). In atheroscelrosis Apo B100 lipoproteins become oxidatively modified. This modification stimulates different biological processes including innate immune system-mediated inflammation which induce a cascade of pathological events than culminate in atherosclerotique plaques (46). In AMD, the following evidence supports the "response to retention" hypothesis:

- Apo B100-containing lipoproteins accumulate in Bruch's membrane in the same location as basal deposits in drusen;
- oxidatively modified proteins and lipids are present in Bruch's membrane and RPE inducing a pathologic phenotype to RPE cells (47); and
- the accumulation of inflammatory mediators whitin drusen and basal deposites indicates a role for the innate immune response (48).

Oxidized lipoproteins can trigger complement activation (46). CD36 is the major receptor implicated in uptaking the oxidized low density lipoproteins and is expressed also in RPE cells. It has been suggested that CD36 may have a role not only in the clearance of oxidized lipids from Bruch's membrane (49) but also in the subsequently inducing an immune response (50).

In addition to aforementioned facts, the presence of matrix matalloproteinases (MMPs) in higher concentration in the Bruch's membrane and RPE cells, especially MMP-2 and MMP-9 indicate to more similarity between atherosclerotic plaque and AMD lesion formation. It is known that MMP-2 and MMP-9 are implicated in the degradation of extracellular matrix components which can lead to plaque destabilization and rupture and subsequent future cardiovascular events, especially acute myocardial infarction (AMI) (51).

Chronic inflammation seems to be a causative factor for the development of AMD. Chronic inflammation results in endothelial dysfunction and facilitates the interactions between modified lipoproteins, monocyte-derived macrophages, T-cells and normal cellular elements of the retinal arterial wall (52). Macrophages are often seen in the area of geographic atrophy and are apparently phagocytosing pigment debris, as seen by electron microscopy or immunohistochemistry methods (44,53). Macrophages have been documented both morphologically and functionally in neovascular AMD (54). Activated macrophages and microglia may secrete chemokines and citokines, causing further cellular damage, Bruch's membrane degradation and angiogenesis (55).

The human eye is known to produce significant quantities of 7-ketocholesterol and related substances as a direct result of photoreceptor function. In atherosclerosis oxysterols contribute to the conversion of macrophages into foam cells (56). 7-ketocholesterol has recently been found to be localized in deposits within the choriocapilaris and Bruch's membrane of aging monkies (57). Oxysterols have cytotoxic and inflammatory properties on RPE cells inducing reactive oxygen species generation, glutathione depletion, and reduced mitochondrial membrane potential inflammation through activation of NFkB and eventually apoptotic-mediated cell death in cultured RPE cells (58).

Recently, a strong association between the Y402H single-nucleotide polymorphism in the complement factor H (*CFH*) gene and AMD was found in 3 clinic-based case control studies (59), and in a longitudinal population based study (60). Complement factor H is an essential regulator in the complement system. It activates C3b and functions as an activation inhibitor of the alternative complement pathway (61). This single-nucleotide polymorphism is located in a region that contains the binding sites for heparin and CRP. Complement factor H binds to CRP, which may help inhibit the CRP-dependent alternative pathway activation induced by damaged tissue (61). Complement factor H tends to prevent the assembly of complement complex in the arterial intima (62). It has been suggested that allele-specific changes in activities of the binding sites for heparin and CRP modify the protective action of complement factor H (63). Complement-related damage to choroidal vessels might lead to wet AMD (11). It is possible that reduction of CRP levels might lower the risk of AMD. Some recently published papers indicated that CFH binds to the denaturated rather than native

CRP thus casting some doubt upon this link between CFH and CRP (64). It is also possible that persistent chronic inflammation that is a byproduct of attenuated complement-inhibitory activity may occur in those individuals with the risk-conferring CFH SNP Y402H and that this pro-inflammatory state, rather than impaired binding by CFH, leads to CRP accumulation in AMD retina. Alternatively, the role of CFH in AMD might be completely independent of CRP. Without a doubt, further studies are necessary to dissect the role, if any, of the CFH Y402H SNP in AMD pathogenesis. Deangelis and coworkers stated that there was a clear genetic influence on AMD, and the loci 1q33 (CFH) and 10q26 (PLEKHA1/ARMS2/HTRA1) were the most strongly associated with AMD, but the variation of these genomic regions alone were unable to predict disease development with high accuracy (65). Lederman *et al*. demonstrated that neovascular AMD was associated with altered gene expression in peripheral white blood cells that was not underlined by the major risk single nucleotide polymorphisms, and suggested that such altered expression may potentially serve as a biomarker for the disease (66). Increased levels of annexin A5 (ANXA5) mRNA transcripts were also found in the WBC of patients with AMD. ANXA5 which plays a role in the regulation of blood clot has been found in atherosclerotic plaques and is proposed to have and anti-inflammatory functions (67). Interestingly, other annexins were previously identified in drusen (68). Recent studies have revealed profound developmental consequences of mutations in genes encoding proteins of the lectin pathway of complement activation, a central component of the innate immune system. Apart from impairment of immunity against microorganisms, it is known that hereditary deficiencies of this system predispose one to autoimmune conditions. Polymorphisms in complement genes are linked to, for example, atypical hemolytic uremia and age-dependent macular degeneration. The recently discovered lectin pathway is less studied, but polymorphisms in the plasma pattern-recognition molecule mannan-binding lectin (MBL) are known to impact its level, and polymorphisms in the MBL-associated serine protease-2 (MASP-2) result in defects of complement activation (69).

## Association of CRP with AMD

Several recent clinical studies suggest close association between serum CRP and ocular vascular disorders related to AMD. One recent study (70) demonstrated a close positive association between CRP and cholesterol levels (especially total cholesterol, LDL- and non-HDL cholesterol levels) in patients with AMD. AMD patients who had higher values of total-, LDL- and non-HDL-cholesterol values had also higher CRP values. This group of investigators succeeded to demonstrate a significant association between incidence of AMD and CRP levels, especially between occurrence of AMD and CRP levels higher than 3 mg/L. Several recent clinical studies reported that patients with the highest quartile of CRP (over 6.5 μg/mL) are at high risk of AMD (5,71). In addition, more than threefold higher incidence of AMD was found in women with serum CRP levels exceeding 5 μg/mL (5,71). The concentrations of CRP used in the study of Nagaoka (30) (0.7 and 7 μg/mL) covered the physiological and pathophysiological ranges, and only high level of CRP exhibited inhibitory action in endothelium-dependent vasomotor function. It appears that CRP levels known to predict cardiovascular events produce adverse effects on endothelial function in the retinal microvasculature. C-reactive protein (CRP) is an inflammatory marker known to be associated with cardiovascular disease, and a link between AMD and CRP has been suggested. Hong *et al*. (72) in his systematic review summarize the currently available evidence from clinic-based and population-based studies investigating this association. Their meta-analysis shows that high serum levels (> 3 mg/L) of CRP are associated with a two-fold likelihood of late onset AMD, compared to low levels (< 1 mg/L).

De Jong *et al*. (73) showed, in the Rotterdam study, the existence of a small significant association between log CRP levels and AMD incident. Kikuchi *et al*. (74) demonstrated the trends of the increased risk of disease with the increase of CRP, which were statistically significant for both polypoidal choroidal vasculopathy (PCV) and neovascular AMD. The Rotterdam study (38) found that elevated baseline levels of high sensitive CRP (HsCRP) were associat-

ed with the development of early and late AMD in the large population-based cohort. Boey *et al*. (75) demonstrated no associations between CRP and AMD or cataract in general population of the Asian people, while higher CRP was associated with AMD in individuals without diabetes.

## Possible mechanism for CRP dependent-oxidative stress and lipid disorder

The inflammatory reaction is an important source of the oxygen-free radicals. Large amounts of superoxide radicals are secreted by activated phagocytic leukocytes, and also formed as by-product during biosynthesis of leukotrienes and prostaglandins and formation of lipid peroxides.

Proinflammatory cytokines play a central role in mediating the cellular and physiological responses. Non-enzymatic oxidative modification mediated by reactive oxygen species transforms low density lipoprotein (LDL) to an atherogenic molecule (E-LDL) that activates complement and macrophages and is present in the early atherosclerotic lesions and drusen. E-LDL accumulates in the human vascular smooth muscle cells (VSMC) where promotes angiotensin type 1 receptor ($AT_1$-R) upregulation and stimulates VSMC migration proliferation and neointimal formation while concomitantly increasing reactive oxygen species (ROS) production. A growing body of evidence implicates CRP as a direct mediator of endothelial dysfunction. CRP directly upregulates endothelial cell adhesion molecules: ICAM-1, VCAM-1 and E-selectin, which play a key role in facilitating the leukocyte-endothelial interaction. CRP also promotes the release of MCP-1 a key chemoatractant chemokine which facilitates leukocyte transmigration through the endothelium (11,76). Recent studies suggest that CRP also promotes nuclear factor (NF)-κB upregulation in endothelial cells (77). In that way CRP functions as an active participant in lesion formation and hence is directlly linked to atherosclerosis.

CRP is capable of generating the TEMPOL-sensitive superoxide in the endothelial layer of the retinal arterioles. This finding is consistent with recent evidence showing that CRP can increase the production of superoxide in cultured human aortic endothelial cells and in porcine coronary arterioles (78). Clinical study of Fichtlscherer (79) reported that the increase of oxidative stress and the reduction of NO bioavailability were closely related to elevation of plasma CRP in patients with the coronary artery disease. The findings of Nagaoka (30) suggested that detrimental effects of CRP could also affect the ocular circulation and might partially contribute to development of the retinal vascular disease.

It has been demonstrated that CRP, in concentration known to predict vascular disease, directly inhibits the endothelium-dependent NO-mediated dilation of the isolated porcine retinal arterioles. The mechanism underlying the acute effect of CRP involves the activation of p38 kinase and the production of superoxide by vascular NAD(P)H oxidase. Recent clinical studies have demonstrated that statins are beneficial by preserving the endothelial function, possibly through the inactivation of the RhoA/Rho-kinase pathway and reduction of the oxidative stress. Since the impaired endothelium-dependent NO-mediated dilation is a key feature of the early vascular events, it is clear that CRP is not only an inflammatory marker but also a mediator of development of vascular disorders in the retinal circulation. Reductions in inflammation and oxidative stress or inhibition of RhoA/Rho-kinase activity (for example by statins) have been reported to improve endothelial function (80,81).

It has been demonstrated that human CRP could be bound with highest affinity to phosphocholine residues, but it also binds to a variety of other autologous and extrinsic ligands, and it aggregates or precipitates the cellular and molecular structures bearing these ligands. Autologous ligands include native and modified plasma lipoproteins, damaged cell membranes, a number of different phospholipids and related compounds, small nuclear ribonucleoprotein particles, and apoptotic cells. Binding of CRP to lipids, especially lecithin (phosphatidyl choline), and to plasma lipoproteins has been documented to be the first step in gen-

eration of foam cells and atherogenesis (82). It has been also demonstrated that aggregated, but not native, non-aggregated, CRP selectively binds only LDL and some VLDL particles from the whole serum. Native CRP does bind to oxidized LDL and to partly degraded LDL, as found in atheromatous plaques (83). When aggregated or bound to macromolecular ligands, human CRP is recognized by C1q and potently activates the classical complement pathway, engaging C3, the main adhesion molecule of the complement system, and the terminal membrane attack complex, C5–C9. Bound CRP may also provide secondary binding sites for factor H and thereby regulate alternative-pathway amplification and C5 convertases (84).

## Conclusion

According to recent studies, CRP is definitely not only the inflammatory marker but also a mediator for development of the vascular disorders in the retinal circulation. The results obtained from the present studies may help our understanding the pathogenesis of the retinal vascular disease associated with high levels of CRP. Since there is no cure for AMD, prevention is the first approach to reduce vision loss. Control of modifiable risk factors such as smoking, hypertension, hyperlipoproteinemia, oxidative stress and chronic inflammation could reduce the risk of developing AMD.

## Vacabulary

- The **choroid**, also known as the *choroidea* or *choroid coat*, is the vascular layer of the eye, containing connective tissue, and lying between the retina and the sclera (85).
- **Retina** is a light-sensitive tissue lining the inner surface of the eye. The optics of the eye create an image of the visual world on the retina, which serves much the same function as the film in a camera (86).
- The **pigmented layer of retina** or *retinal pigment epithelium (RPE)* is the pigmented cell layer just outside the neurosensory retina that nourishes retinal visual cells, and is firmly attached to the underlying choroid and overlying retinal visual cells (87).
- The **fovea centralis**, also generally known as the *fovea*, is a part of the eye, located in the center of the macula region of the retina. The fovea is responsible for sharp central vision (also called foveal vision), which is necessary in humans for reading, watching television or movies, driving, and any activity where visual detail is of primary importance (88).
- The **macula** or *macula lutea* (from Latin *macula*, "spot" + *lutea*, "yellow") is an oval-shaped highly pigmented yellow spot near the center of the retina of the human eye, and acts as a natural sunblock (analogous to sunglasses) for this area of the retina. The yellow colour comes from its content of lutein and zeaxanthin (89).
- The **sclera** (from the Greek *skleros*, meaning hard, also known as the *white* or *white of the eye*, is the opaque (usually white, though certain animals, such as horses and lizards, can have black sclera), fibrous, protective, outer layer of the eye containing collagen and elastic fiber (90).
- **Bruch's membrane** is located between RPE and choroid and it represents a semipermeable filtration barrier through which major metabolic exchange take place (91).

**Acknowledgment**

This work was supported by the Ministry of Science of Serbia through contract No175036.

**Potential conflict of interest**

None declared.

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## Uloga CRP i upale u patogenezi makularne degeneracije povezane sa starošću


**Sažetak**

Makularna degeneracija povezana sa starošću (engl. *age-related macular degeneration*, AMD) kompleksna je, degenerativna i progresivna bolest koja uključuje višestruke genetske i okolišne čimbenike te može rezultirati ozbiljnim gubitkom vida. Etiologila AMD još se ne razumije u potpunosti. Postoje mnoge teorije koje ističu mehanizme oksidativnog stresa, promjene nalik na aterosklerotske promjene, genetske predispozicije i upalu. Najnovija klinička istraživanja proučavaju važnu ulogu upale i C-reaktivnog proteina (CRP) u patogenezi AMD. Postoji veliki broj dokaza koji ukazuju na povezanost CRP-a s endotelnom disfunkcijom, oksidativnim stresom i stvaranjem reaktivnih kisikovih spojeva kao i s poremećajem lipidnog statusa kod bolesnika oboljelih od AMD. Prema navodima novijih istraživanja CRP definitivno nije samo upalni biljeg, već i posrednik u razvoju vaskularnih poremećaja u retinalnoj cirkulaciji. Rezultati dobiveni nedavno provedenim istraživanjima mogu pomoći pri razumijevanju patogeneze retinalne vaskularne bolesti povezane s visokom koncentracijom CRP-a.



**Ključne riječi**: makularna degeneracija povezane sa starošću; C-reaktivni protein; upala