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Biomarkers of Oculopathy in Hereditary Transthyretin Amyloidosis

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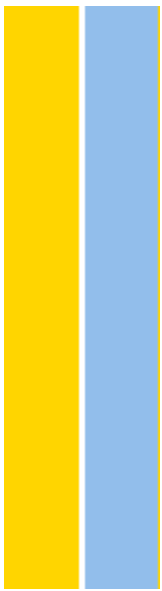

INSTITUTO DE **CIÊNCIAS BIOMÉDICAS ABEL SALAZAR**

Biomarcadores da Oculopatia na Polineuropatia Amiloidótica Familiar

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Aos meus amigos, que caminham comigo todos os dias.

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Resumo

Introdução: O prognóstico da Polineuropatia Amiloidótica Familiar mudou por completo com a adoção terapêutica do transplante hepático, estabilizadores da transtirretina e silenciadores de gene, que permitem hoje travar satisfatoriamente a progressão da doença sistémica. Ainda assim, nenhum tratamento demonstrou eficácia em conter a progressão da oculopatia, o que se torna particularmente relevante agora que o aumento da esperança média de vida dos pacientes conduz a que virtualmente todos eles venham a desenvolver doença ocular [1,2].

Objetivos: Esta dissertação pretende rever a literatura existente com o objetivo de identificar possíveis biomarcadores de oculopatia na amiloidose associada a transtirretina, com o intuito de monitorizar a progressão da doença, identificar doentes em risco acrescido de complicações, avaliar modalidades diagnósticas e/ou verificar a eficácia de futuros tratamentos oculares.

Desenvolvimento: Vários biomarcadores têm sido apontados como potencialmente úteis na monitorização da amiloidose ocular. Com efeito, anormalidades vasculares na conjuntiva podem ser usadas para monitorizar a doença sistémica e a perda de função neurológica [3]. A íris denteada precede o glaucoma, pelo que, havendo alterações na morfologia pupilar, a pupilometria e o flare da câmara anterior podem determinar olhos em risco de complicações [4,5]. Opacidades vítreas são uma forma comum de amiloidose ocular, necessitando de cirurgia, que restaura a acuidade visual, mas agrava o risco de glaucoma e retinopatia vascular. Como tal, estas podem ser importantes para determinar o correto tempo cirúrgico e vigilância necessária, através de medições da Pressão Intraocular, de documentação angiográfica e dos níveis séricos e oculares de VEGF [6–8]. Angiograficamente, um padrão difuso de hipercianescência está associado a doença neurológica mais severa [9,10] e depósitos em forma de agulha na membrana limitante interna a um risco maior de recorrência de opacidades vítreas[10]. Adicionalmente, alterações como o aumento da zona avascular da fóvea no OCT-A também estão associadas a doença mais severa e tardia [11].

Conclusões: É importante ter consciência das manifestações da oculopatia amiloidótica, visto que alterações como opacidades vítreas, glaucoma e retinopatia podem levar a perdas importantes de visão. Assim, o desenvolvimento de biomarcadores é essencial para melhor monitorizar a progressão da doença, sendo que mais estudo será necessário para expandir o presente conhecimento e determinar formas de corretamente interpretar cada biomarcador.

Palavras-Chave: Amiloidose ATTR, Amiloidose Ocular, Biomarcadores, Pressão Intraocular, Tomografia de coerência ótica, Angiografia, Retinografia, Córnea, Íris, Cristalino, Vítreo, Retina.

Abstract

Introduction: Liver transplantation, transthyretin stabilizers, and gene silencers have been shown to successfully halt systemic disease progression in familial amyloidosis. Unfortunately, no treatments have been found to prevent the progression of ocular disease. This is particularly relevant now that increased life expectancy due to effective systemic treatment has made it so essentially all patients with hATTR will live long enough to develop ocular manifestations[1,2].

Objectives: The purpose of this dissertation is to review existing literature to determine what could be used as a biomarker for ocular disease progression in TTR amyloidosis, further studying the possibility of anticipating patients with an increased risk of complications, evaluating diagnostic modalities, or assessing the effectiveness of future treatments for the eye.

Development: Several biomarkers have been theorized to be helpful ways to monitor ocular disease progression, which this review aimed to explore. Abnormal Conjunctival Vessels could be used to monitor systemic disease and neurological impairment[3]. Scalloped Irises are known to precede glaucoma. As such, if pupillary rim changes are present, pupillometry and anterior chamber flare could be used to determine eyes at risk for glaucoma [4,5]. Vitreous opacities are a common form of ocular amyloidosis and often require surgery, which restores visual acuity, but worsens glaucoma and retinopathy. As such, vitreous opacities could be used as a biomarker to determine the best timing for surgery and the follow-up needed, with close IOP monitoring, angiographic documentation, and, potentially, VEGF level determination[6,12]. Imaging could also be important, with a diffuse hypercyanescence pattern on angiography associated with more severe neurological disease[9]. OCT showing needle-shaped deposits in the ILM has been associated with a higher recurrence of vitreous opacities [10] and changes like the increase of the foveal avascular zone as seen on OCT-A are more prevalent in later, more severe stages of the disease [13].

Conclusion: Clinicians must be aware of potential ocular manifestations in amyloidosis patients to detect them timely, since alterations like vitreous opacities, glaucoma, and retinal vasculopathy can lead to significant visual impairment and need therapeutic approaches. As such, the study and development of biomarkers is essential for monitoring disease progression. Further studies are needed to expand current knowledge and establish guidelines for interpreting each biomarker.

Keywords: ATTR Amyloidosis, Ocular Amyloidosis, Biomarkers, Intraocular pressure, Optical coherence tomography, Angiography, Retinography, Cornea, Iris, Lens, Vitreous, Retina.

List of Abbreviations

hATTR – Hereditary Transthyretin Amyloidosis

TTR – Transthyretin

siRNA - Small interfering RNA

ASO – Antisense oligonucleotides

V30M – Valine 30 Methionine

ATTRV30M – V30M mutated Transthyretin Amyloidosis

ACV – Abnormal Conjunctival Vessels

IOP – Intraocular Pressure

VEGF – Vascular Endothelial Growth Factor

OCT – Optical Coherence Tomography

OCT-A – Optical Coherence Tomography Angiography

ILM – Internal Limiting Membrane

RPE – Retinal Pigment Epithelium

CPE – Ciliary Pigment Epithelium

BRB – Blood Retina Barrier

TBUT – Tear Break-Up Time

CMM – Corneal Confocal Microscopy

ODSI – Ocular Disease Surface Index

CFS – Corneal Fluorescein Staining

BCVA – Best Corrected Visual Acuity

PPV – Pars Plana Vitrectomy

RAA – Retinal Amyloidotic Angiopathy

CAA – Choroidal Amyloidotic Angiopathy

Table of Contents

List of Tables/Figures

Methodology

A thorough search for scientific articles was made on PubMed, Web of Science, and Embase, focusing on retrospective and prospective studies, review articles, case series, and case reports. The articles analyzed were in the English and Portuguese languages and were obtained up to February 2025, with preference given to those recently published, if applicable.

The electronic databases were searched using the following 134 keyword combination: “hereditary amyloidosis” [tw] OR “ATTR amyloid*”[tw] OR “V30M amyloid*”[tw] OR Val30Met amyloid*”[tw] OR “Transthyretin Amyloid”[tw]. “Eye diseases/diagnosis” [Mesh] OR “Eye diseases/genetics” [Mesh] OR “Eye diseases/surgery”[Mesh] OR “ocular involvement”[tw] OR “eye manifestation*”[tw] OR “eye involvement”[tw] OR “ocular manifestation*”[tw]. “Biomarker*” [tw] OR “anterior segment”[tw] OR “posterior segment”[tw] OR “intraocular pressure”[tw] OR “IOP”[tw] OR “OCT”[tw] OR “optical coherence tomography”[tw] OR “angiography”[tw] OR “fundus autofluorescence”[tw] OR “retinography”[tw] OR "cornea"[tw] OR "iris"[tw] OR "lens"[tw] OR "vitreous"[tw] OR "retina"[tw].

Abstracts were then screened for relevance, duplication, and, if selected, efforts were made to procure full-text manuscripts. If electronic copies were found to be unavailable, searches were made in the library of Centro Hospitalar Universitário de Santo António. References of the included articles were then screened to find more articles of interest. Articles were excluded if no full-text publication was retrieved, if articles were not in either the English or Portuguese languages, and if case reports or series described non-TTR types of amyloidosis.

After this screening process was made, 94 articles were found relevant and included in this review.

Introduction

Amyloid

The term “Amyloid” was first used in medicine by Virchow to describe a specific tissue reaction of the *corpora amylacea* of the brain to iodine tincture. Later, amyloid deposits were demonstrated to have increased green, yellow, and orange birefringence under light microscopy, which was further increased after Congo red dye staining [14] . Amyloid deposits owe this characteristic to their similar peculiar tinctorial and physical conformation in beta sheets, which deposit parallel to the direction of the amyloid fibril and are further stabilized by electrostatic attractions[15–17] . This occurs despite the very differing biochemical compositions of these proteins, of which at least 36 distinct prototypes have been described in humans [18] . Each fibril protein type is named by adding a shortened version of the suffix to the precursor protein name. For example, AL amyloidosis has the letter “A” followed by the letter “L”, since the amyloid fibrils are derived from immunoglobulin light chains [2,19].

Amyloidosis

“Amyloidosis” is used to describe a group of heterogeneous diseases characterized by the synthesis of proteins that, as a consequence of the misfolding process explained above, form insoluble fibrillary extracellular aggregates with specific tinctorial characteristics, which then deposit in certain tissues, resulting in progressive organ damage [2].

Amyloidosis can be grouped into localized, in which amyloid deposits are essentially happening within one tissue or organ, or systemic, with multiorgan involvement. As for the etiology of amyloidosis, they are further divided into acquired (primary and secondary) and hereditary [1].

Localized amyloidosis are characterized by in situ production of the precursor protein. Such is the case, for example, in Alac amyloidosis, characterized by the isolated corneal deposition of lactoferrin, causing symptoms related to corneal involvement [1,20]. As for systemic amyloidosis, production of the precursor protein is generally systemic and/or released into the plasma, having the potential to deposit in any given tissue. However, deposition patterns for different types of systemic amyloidosis can be predicted within a certain interindividual variability, which can be explained by inherent tropism for deposition of the amyloid fibrils in certain organs depending on their precursor, the mechanisms of which are not yet entirely understood [1,21].

An additional mention could be made to certain incredibly rare amyloidosis types that cause intracellular deposition of fibrils, which form deposits identical to those found extracellularly [1,22]. Some of the most common types of systemic amyloidosis and other localized phenotypes with affinity towards the eye are outlined in Table I.

AL amyloidosis is the most common form of amyloidosis and is derived from immunoglobulin light chain molecules of the kappa or lambda types that are commonly associated with plasma cell dyscrasias, usually presenting with renal and cardiac involvement through nephrotic syndrome and congestive heart failure [1,2]. Danmacco et al found that ocular involvement of variable phenotypes was detected in 18 out of 111 patients with primary systemic AL amyloidosis [2], which manifested as recurrent periocular ecchymosis, in its most severe form termed “raccoon eyes”, motility defects, with diplopia or ophthalmoplegia of one or more extraocular muscles, and an orbital mass (named “amyloidoma”) which frequently required surgical excision [21].

AA amyloidosis or reactive secondary amyloidosis is derived from serum amyloid A (SAA), an acute phase reactant, whose fragment undergoes amyloidogenesis. AA amyloidosis is, therefore, associated with recurrent infections or chronic inflammatory diseases, with a particular association with an autosomal recessive disorder, familial Mediterranean Fever. In AA amyloidosis, ocular involvement has been found to correlate pathogenetically to the underlying disease, such as episcleritis in rheumatoid arthritis or retinal vasculitis in Behçet’s disease, not being therefore as often caused by the amyloid protein deposition itself [1,2,21,23].

Transthyretin amyloid defects are associated with various types of systemic amyloidosis. On the one hand, hATTR, even if quite rare, is one of the most common causes of hereditary amyloidosis, for which more than 130 TTR point mutations have been reported in literature [1,20]. On the other hand, Senile systemic amyloidosis or wild-type TTR amyloidosis, characterized by the deposition of non-mutated (wild-type) transthyretin, is incredibly prevalent in older patients, being the most common cause of cardiac amyloidosis, present in up to 25% of all autopsied bodies over the age of 80, even if, most of the time, it was present with little or no clinical significance [1,21].

Transthyretin

Transthyretin, initially known as prealbumin, is a tetrameric plasma protein, in which each of the subunits is made up of 127 amino acids. The TTR molecule itself contains a lot of beta-pleated sheet structures, a known property commonly associated with proteins that polymerize into amyloid fibrils [12,21]. Transthyretin functions as the transporter of about 15-20% of serum thyroxine and has an essential role in the transport of Vitamin A, through the formation of a complex with retinol binding protein [1,12,21,24]. 90% of transthyretin in the serum is produced in the liver, with the remainder of its production attributed to the retinal pigment epithelium (RPE), ciliary pigment epithelium (CPE) and to the choroid plexus, where it has been associated with a neuroprotective function [1,21,25–27]. Point mutations on TTR are therefore linked with hereditary amyloidosis, predominantly of the neuropathic and cardiac types [21].

TTR Amyloidosis

ATTR was first described in 1952 by Corino de Andrade as a form of peculiar hereditary autosomal dominant sensorimotor polyneuropathy initially found around the fishermen community of Póvoa de Varzim, on the northern coast of Portugal. This disease was described as a polyneuropathy that affected predominantly the lower limbs, associated with severe autonomic dysfunction, with the first causative mutation being identified as a substitution of valine for methionine in position 30 of the transthyretin protein (V30M), still the most prevalent in Portugal [1,24,28,29]. However, it is today believed that, if nonendemic areas are accounted for, VAL122Ile may be the most prevalent worldwide [30].

Nowadays, hATTR is known as a rare autosomal dominant disease, considered endemic to Portugal, Japan, and Sweden, with the global prevalence being estimated at around 40.000 affected individuals [31,32]. The median age for symptom onset in Portugal appears to be around the mid-30s, with 80% of carriers developing symptoms before the age of 40, appearing earlier in men than in women [1]. Portuguese ATTRV30M is coined as being “early-onset”, with symptoms developing before the age of 50 and typically having a complete penetrance. It is also associated with a more aggressive, rapidly progressive disease [1,33]. In Sweden, patients are described as having a typically “late-onset disease”, usually over the age of 50, with a similar age for symptom onset being found in nonendemic areas for hATTR [1,24,32–34]. As such, a growing consensus seems to point to a skewing of epidemiological results, since published estimates of disease milestones tend to be researched in endemic areas, out of which disease seems to have a much later onset, at around 61.5 (±11.5) years, whereas traditionally, disease onset has been described to occur by the age of 50. [24,35,36].

Both in Portuguese and Swedish patients, familial anticipation of symptom onset has been described [37]. The reported mean time from symptom onset to death in untreated individuals is 10–15 years for all disease phenotypes of ATTR Polyneuropathy [35,36].

Clinical Manifestations

ATTR polyneuropathy typically manifests with hypoesthesia and dysesthesia, with inevitable progression to motor involvement with paresis and muscle paralysis, which typically both start in the lower limbs and progress upwards [1,24]. Dysautonomia is also frequent and established early in the disease cycle, with cardiovascular and gastrointestinal involvement, in parallel with sphincter incontinence and particularly severe early erectile and bladder dysfunction [38,39]. In patients with late-onset disease, neuropathic pain seems to be a common early presentation [40–43]. Cardiovascular alterations tend to appear together with dysautonomia, typically consisting of orthostatic hypotension and supine hypertension, with only very few cases progressing to clinically significant heart failure [44,45]. Particularly relevant nowadays is the ophthalmological impairment of ATTR polyneuropathy, which has become increasingly prevalent as disease treatment both improves the life expectancy of patients and has been ineffective in halting the progression of ocular disease [46–49].

Treatment of hATTR

As was previously mentioned, about 90% of transthyretin is produced in the liver, with the rest owing its production to the retinal and ciliary pigment epithelium cells and the choroid plexus of the brain [48,50–52].

This made liver transplantation a logical way of removing circulating TTR protein and halting systemic disease progression, which has been performed to great success [1,2,53]. The first liver transplantation as treatment of ATTR polyneuropathy was performed in Sweden in 1990 and showed promising results, with survival rates of over 50% in 20 years [50,54,55]. The adoption of this procedure worldwide led, in turn, to a big increase in the waiting time for liver transplantation in endemic areas, which then led to the adoption of domino transplantation, which consisted in the usage of livers explanted from TTR amyloidosis patients, which, apart from the production of mutated transthyretin, were functionally normal, for patients with a more reserved prognosis (usually elderly patients with alcoholic or infection-related cirrhosis or hepatocellular carcinoma), whose life expectancy would in theory be shorter than the time needed for symptom development related to amyloid deposition [56,57].

Many attempts have since been made at developing effective pharmacological treatments that would prevent either amyloid fibril formation or its deposition. Of these attempts, TTR stabilizers such as diflusinal (an NSAID) [58,59] and particularly Tafamidis, a molecule with high tropism for the connection site of thyroxine in the TTR molecule, showed promising results in decreasing amyloidogenesis and halting systemic disease, having since become a mainstay of ATTR treatment [59,60]. Gene silencing, in the form of small interfering RNAs (siRNAs) and antisense oligonucleotides (ASO), is a very promising therapeutic modality in TTR amyloidosis. These drugs use lipid nanoparticle carriers to bind to liver mRNA and reduce TTR production [61–64]. On one hand, SiRNA molecules like Partirisan have been shown to reduce serum levels of mutated TTR by up to 80%, allowing for disease control rates that recent studies suggest are even better than those achieved by tafamidis [65,66]. On the other hand, ASOs like Inotersen are presently showing great results in improving the quality of life of patients in recent clinical trials [67,68]. These new modalities are especially interesting since they are the first treatment option that effectively intervenes in the pathophysiological process of amyloid fibril formation [59,65,69].

Liver transplantation and pharmacological treatments such as tafamidis and gene silencers have, therefore, dramatically increased the overall life expectancy of familial amyloidosis patients. Despite this, it seems none of these treatments have been effective in stopping ocular progression, with the same levels of mutated TTR being found in the aqueous humour of treated and untreated patients [48,49,70,71]. This is owed to the fact that Plasma TTR cannot cross the blood retina barrier (BRB), which means ocular disease is likely to develop due to local production of mutated transthyretin in the Retinal or Ciliary Pigment Epitheliums [46,52,71]. In fact, Beirão et al demonstrated no amyloid deposition in ocular tissues in domino liver recipients [52], further concluding that the ophthalmological follow-up of ATTRV30M patients should be the same, regardless of liver transplant having been performed [72].

As such, not only does ocular disease seem to progress independently from systemic disease, it also appears to progress asymmetrically, with each individual eye functioning as its own closed system [1,47,72,73].

Ocular Manifestations

Clinically significant ocular amyloidosis usually develops late in the disease cycle and was historically not a particularly common finding, but that has been changing since systemic treatment has drastically increased the life expectancy of patients. Recent literature now estimates the prevalence of ocular involvement in up to 25% of patients, with further suggestions that virtually all of them will present with some form of ocular involvement given time for the disease to progress [53,72,74].

The most prevalent findings of oculopathy were determined to be increased tear break-up time (TBUT) and an abnormal Schirmer test [72], suggesting *keratoconjunctivitis sicca* to be a common and early finding, associated with a complex physiopathology in which a reduction in tear quality and production is present due to amyloid deposition in the ciliary gland and neuropathy of the parasympathetic fibers of the VII cranial nerve [1,72,75–77]. This happens in parallel to a reduction in corneal sensitivity due to the local accumulation of amyloid in the epithelium and stroma [78]. This pathophysiological process, if severe enough, can cause dry eye syndrome, corneal ulcer, or even perforation [1,78,79].

Amyloid deposition in the iris was one of the first discovered features of ATTR oculopathy, happening in a temporal pathological sequence that starts with thin deposits in the pupillary border, eventually progressing to the development of a scalloped iris [72]. Affected eyes have associated light and near reflex alterations and anisocoria due to pupillary border deformity [53]. These findings have been shown to progress despite liver transplant, meaning its development is related to endocular TTR production and deposition [1,28,80].

Additionally, amyloid also deposits in the anterior lens capsule, in places such as the zonules, ciliary processes, and ciliary muscles, leading to a loss of lens elasticity and to autonomic neuropathy. These can lead to accommodation defects, loss of spatial contrast, and early development of cataracts and presbyopia [72,78,81].

Vitreous amyloidosis has also been described by some authors as one of the most frequent endocular manifestations of ATTR amyloidosis [73]. These tend to be bilateral and asymmetric, due to endocular production of mutated TTR in the RPE. Vitreous amyloidosis is caused by the aggregation of amyloid to type 2 collagen, leading to deposits in the vitreous matrix [82]. It is usually described as having a sheet-like/cobweb-like appearance, with an opacity that can be traced from the meshwork into the posterior lens capsule, named *pseudopodia lentis* [83–85].

Val30Met patients tend to present later and less commonly with vitreous amyloidosis than other mutations, such as Arg34Gly or Tyr114Cys [21,82,86], with the latter being associated with vitreous disease in almost all patients [2], sometimes even before systemic disease onset [87]. Recently, the study of such mutations has motivated research into establishing criteria to alert ophthalmologists of when to suspect amyloidosis is present in patients with isolated vitreous opacities [88,89]. Symptoms start with nonspecific alterations such as blurred vision and floaters, leading rapidly to progressive vision loss [21,53,84,85]. Visual Acuity can be satisfactorily restored with *pars plana* vitrectomy [90,91]. However, there is an association between an increased risk of glaucoma development and this surgical intervention, with small-gauge vitrectomy with minimal conjunctiva and scleral scarring being increasingly relevant to facilitate future glaucoma surgery if needed [6]. Additionally, recurrence is frequent, with a second intervention and tight follow-up necessary for maintaining visual acuity [1,21,92,93].

Chronic open-angle glaucoma is the leading cause of irreversible vision loss in these patients, which makes it a serious complication of ATTR oculopathy [78]. Additionally, it tends to be resistant to medical treatment and to progress after and independently from liver transplantation, with up to 80% of patients needing surgical treatment, with mitomycin C-augmented trabeculotomy as the preferential modality [85,87]. Glaucoma appears due to a multifaceted pathophysiological process, comprising conjunctival and episcleral perivascular amyloid deposits leading to increased episcleral venous pressure and consequently an increased intraocular pressure, in parallel to deposits in the trabecular meshwork and pupillary border leading to an obstruction in the aqueous outflow route [1,85,94]. Recently, more and more descriptions of neovascular glaucoma and of glaucoma development after *pars plana* vitrectomy have been reported, further enhancing the knowledge that, with the greater life expectancy achieved by systemic treatment, a tight follow-up of oculopathy in these patients is crucial to prevent irreversible vision loss, with observation of amyloid deposits along the pupil theorized to be a good predictor for the onset of glaucoma, as will soon be discussed [1,6,85,87].

Multiple vascular abnormalities have been found in the eye, with the conjunctiva, retina, and choroid all affected by TTR amyloidosis. Abnormal conjunctival vessels (ACV) are caused by a distal oclusive capillaropathy caused by both deposition of amyloid and some degree of dysautonomia [9]. Conjunctival lymphangiectasia has also been reported [78,95]. ACV can be seen on slit lamp examination in the form of microaneurysms. It is the earliest and most frequent sign of the ocular deposition of amyloid protein [1,53]. However, since conjunctival involvement is due to extraocular production in the liver, true epidemiological analysis is difficult, since a big percentage of patients in endemic areas have now been subjected to liver transplant [72].

Retinal angiopathy is less common than conjunctival disease, but subclinical amyloid deposition in the endothelial layer, compromising both retinal and choroidal vessels, is theorized to be present even before oculopathy onset [53,72,85,96]. Retinal vascular abnormalities on fundoscopic examination can range from microaneurysms, hemorrhages, and cotton wool spots to severe ischemia [1,97]. Rubeosis of the iris and neovascular glaucoma have also been reported [98–100], which are severe complications of this disease for which treatments like pan retinal photocoagulation and intravitreal ranibizumab injections have been studied [101–104], with mixed results.

TTR amyloidosis can therefore have very diverse manifestations, meaning the first approach to screening for ocular involvement is a complete ophthalmological check-up with pupil evaluation, corneal confocal microscopy, BCVA, anterior chamber and fundoscopic examinations, tonometry, and, when needed, visual field tests [1,78]. The increasing life expectancy due to systemic treatment and the rising prevalence and severity of oculopathy in ATTR patients make the study of potential biomarkers imperative, which could later be used for early screening of the disease, amplifying diagnostic modalities, or testing and validating future treatments aimed at the eye [11,78,95].

Biomarkers - Discussion

For the sake of discussion, potential biomarkers will be divided into extraocular and endocular, depending on whether TTR production is mediated by the liver or RPE/CPE, respectively. The latter will be further divided into basic and advanced biomarkers, according to whether evaluation of these is available in all ophthalmological departments or if access to them is more limited.

Extraocular Biomarkers

Conjunctival vessel abnormalities have long been pointed to as a potential biomarker for TTR amyloidosis, since it is most of the time clinically insignificant and has been found to be present in most, if not all, patients very early on in the disease cycle [53,72]. Additionally, vasculopathy is caused by the deposition of mutated transthyretin produced in the liver and not in the RPE, which makes CVA a potentially interesting ocular biomarker for the early screening and monitoring of systemic disease [1,3,53,95]. In fact, in a patient with an ATTRD18E mutation, Patil et al determined that conjunctival vessel abnormalities statistically correlated with the worsening and progression of the systemic disease in a 2-year follow-up period [95]. Additionally, Bunod et al studied a 24-person cohort of ATTR patients with the ATTRS77Y mutation and established a 54% prevalence of conjunctival lymphangiectasia, which then had a statistically significant correlation with patients with both more severe neurological disease and clinically significant amyloid cardiomyopathy [3].

Such findings can lead us to theorize that CVA could be important not only as an ocular biomarker for the progression of systemic disease, but also to detect patients with a poorer prognosis due to more severe systemic involvement [3,95].

Other extraocular biomarkers could be useful to assess systemic disease, like Corneal Confocal Microscopy (CMM), Ocular Disease Surface Index (ODSI), corneal fluorescein staining (CFS), Tear Break-up Time (TBUT) and Schirmer Test, and Best Corrected Visual Acuity (BCVA). CMM, specifically, could be used as a means of providing a sensitive and advantageous way to detect amyloidosis-related denervation on the corneal surface, whose findings could, in theory, be extrapolated to systemic neuropathy [78]. Unfortunately, however, no studies have yet addressed these potential biomarkers, but future elaboration could be deemed warranted.

Endocular Biomarkers

Alterations in endocular biomarkers are dependent on TTR produced by the RPE or CPE, meaning they are independent of systemic disease and are generally asymmetric. As mentioned above, for the sake of discussion, these will be split into basic or advanced, based on their availability in all (or just some) ophthalmology departments. An additional division into anterior and posterior segment biomarkers will be made to associate each one with the production of TTR by either the CPE or RPE.

Basic Anterior Segment Biomarkers

Pupillary morphology, represented by the presence of a scalloped iris, is a significant sign of ocular disease progression. In ATTRV30M patients, glaucoma quickly follows the appearance of a scalloped pupil, meaning that objectifying amyloid deposition in the iris in the form of this finding should lead us to consider it a biomarker for glaucoma development and lead us to increase the frequency of intraocular pressure monitoring [4,5,72]. In fact, the increase in IOP itself, even within values considered normal, could also be useful in monitoring ocular disease progression in all patients, particularly in those following vitrectomy, as will be elaborated shortly [6,72].

Amyloid deposition in the anterior lens capsule has been hypothesized by Beirão et al to be the cause of early onset presbyopia in ATTR patients [72,81]. Additionally, clouding of the anterior lens capsule leads to a loss of spatial contrast, which could cause visual complaints in patients with maintained BCVA [105]. Therefore, this makes anterior lens capsule deposits a potential way to determine progression of disease in the posterior chamber of the anterior segment, having the potential to be a valuable finding in the follow-up of these patients, particularly in subclinical cases of oculopathy [72,81].

Basic Posterior Segment Biomarkers

Vitreous amyloidosis is a cause of severe visual deterioration, even if almost always reversible with pars plana vitrectomy (PPV). PPV should be performed when the disease becomes clinically significant, but the pros and cons of surgery should be weighed, as it seems to accelerate the appearance of secondary glaucoma [6,87] and the worsening of the retinal angiopathy, which has been hypothesized to be caused by the deposition of amyloid in the lumen of small vessels that would previously deposit in the vitreous matrix [20]. Ferreira et al noted that vitreous removal was most important before the establishment of *pseudopodia lentis* and alerted to the importance of removing the ILM, a preferred site for post-vitrectomy amyloid deposition, to decrease chances of recurrence [8]. Additionally, Kakihara et al found that post-surgery IOP control was poor (with survival rates of 0.51, 0.38, and 0.23 at 12, 24, and 60 months, respectively), meaning vitrectomy alone isn't enough to maintain optimal visual functions and presented IOP monitoring as crucial for detecting patients who would benefit from surgery [6]. Therefore, vitreous amyloidosis could be best used as a biomarker in helping determine the best timing for surgery and, coupled with IOP monitoring, helping predict the need for glaucoma intervention.

Retinal angiopathy is an important complication of ATTR, appearing to worsen after vitrectomy, making angiographic documentation and follow-up important after surgery [20]. In fact, Zou et al, whilst studying a family with TTR Ala36Pro amyloidosis, which typically presents with early onset oculopathy with vitreous opacities in the 3rd or 4th decades of life, concluded that ATTR patients have increased levels of serum and vitreous VEGF, a potent angiogenic factor, in comparison to controls. Unfortunately, no studies have yet been made to establish a quantitative relation between a said level of serum and vitreous VEGF and different degrees of oculopathy. If such an association could be made, VEGF levels could become an important biomarker for monitoring the progression of retinal and choroidal angiopathy and neovascularization in patients after PPV [7,101,106].

Advanced Anterior Segment Findings

As was previously mentioned, pupillary rim changes seem to always precede the development of glaucoma and clinically significant retinal angiopathy, which makes detecting them an important way of determining when a patient needs tighter IOP monitoring and angiographic documentation [6,20,72]. Recently, Romano et al were able to demonstrate a correlation between pupillometry findings and disease duration [107]. Additionally, Marques et al studied the eyes of ATTR patients presenting with unilateral scalloped irises and found statistically significant changes in both dilation and contraction velocities, which were later corroborated by Kakihara et al [4,108,109]. Therefore, this makes pupillometry a possibly reliable way of objectifying the presence of advanced ocular amyloidosis, helping determine which patients are at an increased risk of local amyloid deposition and glaucoma [4,107].

Anterior Chamber flare evaluates the aqueous flare, which quantitatively assesses inflammation in the anterior chamber. This, in turn, allows us to infer about the integrity of the Blood Aqueous Barrier (BAB) [110–112]. BAB's breakdown in TTR amyloidosis is hypothesized to be due to a combination of endothelial injury caused by systemic TTR and by the intraocular release of proinflammatory markers caused by ocular TTR production and deposition [113]. In fact, in 28 Val30Met patients, Beirão et al found a statistically significant increase in aqueous flare values in patients with scalloped irises, when compared with patients with normal irises (14.1 ± 2.2 versus 6.5 ± 0.9 pc/ms, respectively, with p<0,001). Additionally, IOP values were compared in these patients, but no significant differences were found (p=0,163). As was already discussed, the presence of a scalloped iris is a predictor of risk for developing glaucoma and should increase IOP surveillance. As such, anterior chamber flare could be an important biomarker for monitoring ocular disease, by staging and detecting glaucoma progression early on in patients with scalloped iris, since statistically significant alterations were found even before differences in IOP values were deemed significant. As such, further studies are warranted to determine predictive values of aqueous flare for glaucoma progression [5].

Advanced Posterior Segment Findings/Imaging

Fundus autofluorescence uses the fluorescent characteristics of the retinal pigment in a noninvasive way, being useful in detecting amyloid plaques, since these have a hyper autofluorescent behavior under certain wavelengths in the retina, choroid, and optic disk [114,115]. This characteristic could be used to detect retinopathy, since, as was described by W Lv et al, amyloidosis patients without retinal involvement did not show changes in fundus autofluorescence [116]. However, further studies are necessary to determine the relevance and best way to use this exam to grade retinal pathology in these patients.

OCT studies of the retinal surface in amyloidosis patients have long described the presence of a needle-shaped Internal Limiting Membrane thickening, particularly in patients subjected to vitrectomy [10,117]. In fact, Kakihara et al found this pattern, which tended to be asymmetric, in almost 70% of patients subjected to PPV. In comparison, no patient without vitreous disease and just 4 out of 31 eyes with vitreous disease that had not yet undergone surgery presented this finding [118]. Therefore, OCT changes could be used to screen for retinal disease and to monitor the recurrence of vitreous opacities in the follow-up of patients subjected to PPV [8,10].

Angiography is already used to detect the presence of retinal amyloidotic angiopathy (RAA) and choroidal amyloidotic angiopathy (CAA) [1,115]. Rousseau et al described the most common angiographic patterns in ATTR patients, grading them to correlate the findings with the patient's neurological impairment (which was evaluated with the Polyneuropathy Disability Score) [9]. CAA was found to be more prevalent than RAA and the latter was further divided into three patterns: punctiform (22%), focal (28%), and diffuse (50%) [9,119]. They further concluded that the diffuse pattern was statistically associated with a more severe neurological impairment. Kakihara et al later corroborated these findings, pointing to higher levels of RAA and CAA being associated with more severe systemic involvement of the disease [115,118,119]. As such, angiography could be used as a biomarker for neurological and systemic disease progression.

OCT-Angiography has long been pointed to as a potential biomarker for ATTR patients, since it is already used for non-ocular pathologies like Alzheimer's disease, due to being a noninvasive way of studying the vascular status of certain tissues [13,120]. When studying ATTR patients, Rinaldi et al objectified significant reductions in the superficial and deep choriocapillary plexus densities, along with lower choroidal thicknesses and lower median choroidal vascularity indexes [11]. Kakihara et al, however, found no statistically significant relation between these findings and systemic disease severity [121]. Later, however, Marta et al determined that AATR patients with oculopathy had lower choroidal thicknesses and vascularity indexes than healthy controls, concluding these were useful parameters for monitoring disease progression [106]. Recently, Marques et al added to this idea by comparing patients with scalloped and non-scalloped pupils using OCT-A and noting that eyes with scalloped irises show an attenuated retinal vascular network with a larger foveal avascular zone and a decreased foveal superficial and deep plexus vascular density, concluding that eyes with scalloped irises have more advanced subclinical angiopathy, further pointing to OCT-A's usefulness and to subclinical angiopathy as a clinical biomarker for more advanced ocular disease [122].

As such, different findings on OCT-A seem to be a potentially interesting way to screen for subclinical ocular disease progression, delineating their potential usage in determining the effectiveness of future treatments for ocular disease, with further research needed to fully conclude if correlations can be made between these findings and systemic disease progression [96,121,122].

Conclusion

ATTR polyneuropathy is a rare, multisystemic and fatal disease inherited in an autosomal dominant manner, caused by the extracellular aggregation and deposition of abnormal transthyretin, a protein essential for the transport of vitamin A. About 90% of mutated TTR is produced in the liver, and treatments such as liver transplant, transthyretin stabilizers, and gene silencers have proven incredibly effective in halting the progression of systemic disease and increasing the life expectancy of patients [1,2]. The remaining 10% is produced in the choroid plexus and in the retinal and ciliary pigment epitheliums, these last two being responsible for the oculopathy found in amyloidosis patients, which is nowadays a significant cause of visual impairment, since most of them live long enough to develop ocular disease. Additionally, no treatments have proven effective in arresting disease progression in the eye, meaning the development of biomarkers to better screen for and monitor ocular disease is essential [1,21,52]. In order to center discussion and better focalize ocular disease, biomarkers mentioned were split into extraocular and endocular, the latter of which were further subdivided into anterior and posterior segments, based on the origin of mutated TTR.

Conjunctival vessel abnormalities, an extraocular biomarker, result from systemic deposition of liver-produced mutated TTR and could be a way to screen for and determine systemic disease progression and neurological impairment [3,95]. Anterior capsule deposition could also be a way of screening for ocular disease in patients presenting with visual symptoms like decreased spatial contrast with maintained BCVA [105].

Pupillary alterations like the scalloped iris precede the development of glaucoma, meaning pupillometry could be an interesting way to screen for endocular disease in eyes in which pupillary rim changes are present, with alterations in these found to be a statistically significant way of determining the risk for developing glaucoma, and serving as an indication to tighten monitoring of IOP levels [4,107,108]. In this context, anterior chamber flare could also be useful, as disruption in the BAB seems to be present with increased flare values even before IOP starts rising, presenting another way of screening for the risk of glaucoma, with further studies needed to determine predictive values of aqueous flare [5].

Vitreous opacities are a significant cause of visual impairment, and surgery is needed most of the time, which restores visual acuity but worsens glaucoma and retinal angiopathy [6,92]. As such, vitreous amyloidosis could be best used as a biomarker for determining the most appropriate timing for surgery, predicting the risk of recurrence of vitreous disease, and the importance of a close post-surgery follow-up of glaucoma (through means such as those mentioned above) and retinal angiopathy [7,106], the latter of which could be screened for through both angiographic documentation and by documenting serum and ocular VEGF levels, a potential way of predicting the progression of retinal disease [7,106].

Imaging could also play an important role in ATTR patients. Angiography has found three distinct patterns of retinopathy, with diffuse hypercyanescence statistically associated with more severe neurological disease. Additionally, needle-shaped deposits on the ILM as seen with OCT could be important in predicting the recurrence of vitreous opacities. OCT-A is the most promising of all, as findings such as an increased foveal avascular zone and a decreased vascular plexus density are more prevalent in later stages of the disease, meaning they could be useful ways to track disease progression or even subclinical angiopathy [9–11].

In conclusion, clinicians must be aware of potential manifestations of ocular disease in amyloidosis patients in order to detect them timely, since alterations like vitreous opacities, glaucoma, and retinal vasculopathy can lead to significant visual impairment and require their own therapeutic approaches. As such, the study and recognition of biomarkers is essential to screen for and determine patients at a higher risk for the development of oculopathy, to anticipate future complications of the disease, and to be a future hallmark for testing the applicability and effectiveness of future eye treatments. This review was qualitative and does not therefore ascertain which of these biomarkers would be most useful, most applicable, or most cost-effective, and future research in this area is needed to compare and develop guidelines for each biomarker's interpretation [1,2,47].

Appendix

Table I: Examples of localized and systemic amyloidosis, their clinical manifestations and inheritance pathways.

Amyloidosis Type and Precursor Protein	Localized or Systemic	Hereditary or Acquired	Major Target Organs and Manifestations
AL (Immunoglobulin Light Chain)	Systemic and Localized	Acquired	Affects multiple organs; manifestations include periorbital purpura, conjunctival and orbital amyloidomas, cardiomyopathy, nephropathy, and neuropathy that spares the central nervous system.
AA (Serum Amyloid A)	Systemic	Acquired	Mainly affects the kidneys (nephrotic syndrome), liver, and spleen; secondary to the chronic inflammation of diseases such as rheumatoid arthritis.
ATTR (Transthyretin, Wild-type & Variants)	Systemic	Hereditary or Acquired	Sensorimotor polyneuropathy with occasional cardiomyopathy and severe dysautonomia. Ocular involvement includes vitreous opacities, retinal angiopathy and open-angle glaucoma.
Aβ2M (β2-Microglobulin, Wild-type & Variants)	Systemic	Hereditary or Acquired	Affects the musculoskeletal system and is primarily seen in patients subjected to long-term dialysis treatments, causing joint pain and carpal tunnel syndrome.
AApoA (Apolipoprotein A-I, II, IV, C-II, C-III Variants)	Systemic	Hereditary	Affects the kidneys, liver, heart, peripheral nerves, larynx, and skin (depending on variant).
ALys (Lysozyme Variant)	Systemic	Hereditary	Mainly affects the kidney, leading to progressive kidney failure.
APrP (Prionprotein, wildtype and variants)	Localized	Acquired	Can manifest as Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, and/or fatal insomnia
APro (Prolactin, Pituitary Tumors)	Localized	Acquired	Associated with prolactinomas and amyloid deposition in the pituitary gland.
AKer (Keratoepithelin, Lattice Corneal Dystrophies)	Localized	Hereditary	Affects the cornea, leading to progressive corneal opacification, visual impairment, and recurrent corneal erosions.
ALac (Lactoferrin, Gelatinous Drop-Like Corneal Dystrophy)	Localized	Hereditary	Affects the cornea, being characterized by severe visual impairment and corneal opacities.

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