DOI 10.5935/0034-7280.20150054

Biomarkers and surrogate endpoints in the glaucomatous optic neuropathy: new developments and a review

Biomarcadores e desfechos substitutos na neuropatia óptica glaucomatosa: novos desenvolvimentos e uma revisão

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ABSTRACT

Glaucoma is a group of progressive optic neuropathies that have in common a slow progressive degeneration of retinal ganglion cells and their axons, resulting in a distinct appearance of the optic disc and a concomitant pattern of visual loss. Biomarkers are characteristics objectively measured and evaluated as indication of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Several biological markers have been implicated with glaucoma, especially genetics, proteomics, autoimmune and other molecular biomarkers, although, most awaits clinical validation. There are clear potential benefits in using biomarkers. Information can be obtained earlier, faster, and less costly. This review summarizes the latest developments and approaches in glaucoma biomarkers and its possible uses in the diagnosis, staging, and as predictors of response to treatment.

Keywwords: *Glaucoma; Optic nerve diseases; Biological makers*

RESUMO

O glaucoma compreende um grupo de neuropatias ópticas progressivas que tem em comum a degeneração lenta e progressiva das células ganglionares e seus axônios, resultando em aparência única do disco óptico e, simultaneamente, um padrão correspondente de perda visual. Biomarcadores são características medidas objetivamente e avaliadas como indicadores de processo biológico normal, processos patológicos ou respostas farmacológicas à uma intervenção terapêutica. Vários marcadores biológicos foram associados com glaucoma, especialmente os genéticos, proteômicos, autoimunes e outros biomarcadores moleculares, embora a maioria ainda necessite de validação clínica. Existem potenciais benefícios em usar biomarcadores. Informações podem ser obtidas mais precocemente, de forma mais rápida e menos onerosa. Esta revisão resume os últimos avanços e métodos em biomarcadores de glaucoma e seu possível uso no diagnóstico, estadiamento e como preditores da resposta ao tratamento.

Descritores: Glaucoma; Doenças do nervo óptico; Marcadores biológicos

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The authoes declare no conflicts of interest

Received for publication 21/08/2014 - Accepted for publication 17/12/2014

Biomarkers are characteristics objectively measured and evaluated as indication of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[1] Although what the marker marks is clearly defined as being intrinsic, the marker itself can be either intrinsic or extrinsic. Intrinsic markers can be physical (clinical or radiographic) or laboratorial (physiological, pharmacological, genetic, biochemical, etc.). An example of an extrinsic marker is cigarette consumption in lung cancer.[2] A surrogate marker or surrogate endpoint has been defined as a biomarker intended to substitute for a clinical endpoint, the latter being a characteristic or variable that reflects how a patient feels, functions, or survives.[1]

Both biomarkers and surrogate endpoints can be used in diagnosing, staging, and monitoring disease, and in determining its response to therapy.

The difference between a biomarker and surrogate endpoint is that a biomarker is a "candidate" surrogate marker, whereas a surrogate marker is a test used, and taken, as a measure of the effects of a specific treatment.[3] Biomarkers are often cheaper and easier to measure than true endpoints and can be measured more quickly and earlier. There may also be ethical issues associated with measuring true endpoints. For example, in paracetamol overdose it is unethical to wait for evidence of liver damage before deciding whether or not to treat a patient; instead, a pharmacological biomarker, the plasma paracetamol concentration, is used to predict whether treatment is required.[2]

Biomarkers can be used at any point in the chain of events that leads from the pathogenesis of a disease to its clinical manifestations, whether at the molecular, cellular, or organ levels. Likewise, a therapy might be developed to tackle any one of these links, in order to try to treat the disease. Any measurement short of the actual outcome could be regarded as a surrogate endpoint biomarker. However, although all surrogate endpoints are biomarkers, not all biomarkers are useful surrogate endpoints.[2]

Surrogate endpoints are used in clinical trials and, as such, it is defined as a response variable for which a test of the null hypothesis of no relationship to the treatment groups under comparison is also a valid test of the corresponding null hypothesis based on the true endpoint.[4] It's use, however, introduces heterogeneous variance and the problem of regression to the mean.

In clinical practice, biomarkers are used frequently and without notice. In a patient with cancer, a clinician measures the time to relapse as a surrogate endpoint for survival time. Ophthalmologists measure intraocular pressure (IOP) instead of loss of vision in patients with glaucoma. Physicians use biomarkers to stage disease (e.g., the number of lymph nodes affected by cancer), in diagnosis (e.g., magnetic resonance imaging, electrocardiography, and autoimmune antibodies), and to monitor the progress of a disease or its treatment (e.g., serum glucose concentration and blood pressure).[2]

The increased sensitivity and the developments of genomic, proteomic, and metabolomic research techniques have caused the potential to identify molecules that may serve as potentially useful markers, including (1) markers for early detection of a disease, (2) markers to predict disease severity, (3) markers to predict the rate of disease progression, and (4) markers to serve as predictors of response to treatment.[5]

Glaucoma is a group of progressive optic neuropathies that have in common a slow progressive degeneration of retinal ganglion cells and their axons, resulting in a distinct appearance of the optic disc and a concomitant pattern of visual loss.[6] It is estimated that glaucoma affects more than 66 million individuals worldwide with at least 6.8 million bilaterally blind.[7] Although not completely understood, it is a multifactorial complex neurodegenerative disease triggered by different factors including mechanical stress due to intraocular pressure, decreased blood flow to retina, reperfusion injury, oxidative stress, glutamate excitotoxicity, and aberrant immune response.

The discovery of clinically useful biomarkers in glaucoma is constantly expanding and includes from genes to proteomic markers, and analyses of serum antibodies to retina and optic nerve proteins. We summarize herein the current knowledge regarding the factors related to the diagnosis, progression, and response to treatment of glaucoma, which have not been definitely established but represent biomarker candidates to be validated. These markers include clinical, genetic, proteomic, autoimmune, and neurodegenerative candidates yet to be corroborated.

Clinical biomarkers

Automated perimetry, IOP, optic disk, and retinal nerve fiber layer are surrogate markers for glaucomatous optic neuropathy used in clinical practice and as outcome measures in research. From another standpoint, biomarkers are biological quantitative measurements that may differentiate suspected disease from healthy individuals, and predict the course of disease, or treatment response. So far, we recognize some of the risk factors for glaucoma, such as elevated IOP, although it is not always present in every patient. A combination of two or more biomarkers such as optic nerve structure, visual function and IOP, is a "biosignature" of glaucoma disease, just as measurements of high density lipoprotein (HDL), low density protein (LDL), and cholesterol have become biosignatures of cardiovascular disease.[8]

Intraocular pressure

A number of randomized clinical trials have convincingly shown that elevated IOP is the leading risk factor for glaucoma development and that IOP reduction can significantly reduce the incidence and progression of the disease. However, IOP can be deceiving in that it is not a defining criterion for primary open-angle glaucoma (POAG). Population surveys show that up to 50% of open-angle glaucoma patients have an IOP of 21 mm Hg or lower.[9,10] Besides, the effect of IOP fluctuation, either diurnal or long-term visit to visit, on the risk of developing POAG is still controversial.[11]

Some investigators propose a joint analysis approach to assess whether variability of IOP as a biomarker is independently predictive of clinical outcomes. Using data from two long-term clinical trials of the efficacy of IOP lowering medication in the prevention of glaucoma (the Ocular Hypertension Treatment Study - OHTS and the European Glaucoma Prevention Study - EGPS), they determined if long-term IOP fluctuation is independently predictive of POAG. A linear mixed model incorporating patient-specific variance describes the trajectory of IOP, and its association with the time to POAG is assessed using both semi-parametric and full parametric survival models. Substantively, the authors results show that IOP variability is independently predictive of POAG

in the OHTS, and the subjects with high IOP fluctuation have an increased risk of developing POAG.[12]

Visual function: automated perimetry

The loss of retinal ganglion cells in glaucoma leads to characteristic visual field defects as evaluated by automated perimetry, although a great amount of axons has to be lost before initial visual field defects can be detected. To patients, visual function may be the clearest marker of glaucoma. There seems to be a clear relationship between visual function and quality of life.[13] Automated perimetry is a particularly useful clinical biomarker to predict disease severity, since the visual fields of apatient immediately give an appraisal about the amount of damage, and also the amount of residual vision that is available before the patient will suffer definite and clear loss of quality of life.

Nevertheless, automated perimetry do have some limitations as a biomarker. Visual field defects are not disease specific. Although visual field defects in glaucoma follows typically a ganglion cell loss pattern, other optic neuropathies do cause ganglion cell loss and visual field defects similar to glaucoma.[14] The visual field index (VFI) has been developed to evaluate progression of glaucoma in time.[15] As a marker to predict the rate of disease progression, automated perimetry is not ideal, since it is usually necessary to perform a number of tests to assess progression. Besides, as a psychophysical test it is dependent on patient response and can be very variable from one exam from another. Automated perimetry is not a useful marker for early detection of a disease since a large amount of ganglion cells must be lost before initial visual field defects can be detected.

Structural measures: optic nerve and retinal nerve fiber layer

Direct assessment of the optic nerve and the retinal nerve fiber layer is of paramount importance in glaucoma diagnosis and progression. Progressive optic disc damage is highly predictive for the development of functional loss in glaucoma. Some changes in the optic disk are typical of the glaucomatous optic neuropathy, however, there is a number of patients with suspicious looking disks in which the structural evaluation per se is not enough for diagnosis. Although ephemeral and not very frequent, disk hemorrhage is a very typical feature in glaucoma patients and almost pathognomonic of glaucoma. It may be a marker of rapid glaucoma progression, in that localized subclinical structural change predisposes to disk hemorrhage, after which subsequent disease progression is accelerated, and recurrent optic disk hemorrhages are related to rapid structural progression of glaucomatous damage.[16] The development of new imaging devices provides better access to view the optic disk and the nerve fiber layer as potential biomarkers. Optic coherence tomography is a useful device to assess the optic nerve, and careful monitoring of the optic disc appearance is important to evaluate glaucoma progression. However, whether changes detected by imaging instruments are associated with future progression remains a key question and the ability to longitudinally evaluate imaging instruments is difficult as the technology is always evolving.

Genetic biomarkers

Family history is a risk factor of glaucoma. In fact, about 20% of glaucoma patients have family history of the disease and the prevalence of open-angle glaucoma increases up to 13.5% among relatives of glaucoma patients indicating an important genetic component.[17,18] Genetic biomarkers might be invaluable tools to identify individuals at risk for disease as well as serving to measure the outcomes of therapies. One drawback is that no all genes can function as biomarkers. In order to have a large effect size the allele frequency has to be low; conversely, a high allele frequency has low effect size. Besides, most gene mutations and polymorphism are population specific; so one particular gene mutation in Europeans descendents may not be implicated in an African population.

Model-dependent linkage analyses using multiplex POAG pedigrees have generated a number of potential loci (*GLC1A*–*GLC1H* and *GLC1L*) however, only three genes have been recognized.

MYOC (myocilin) was the first gene identified from the *GLC1A* locus.[19,20] Myocilin is an extracellular protein of unknown ocular function. Missense mutations account for 3% to 5% of POAG cases.[21,22] The underlying genetic mechanism is possibly gain-of function or dominant-negative effect, since the loss of protein function does not result in glaucoma.[23-25] The disease-associated missense changes reduce the solubility of the protein, causing it to aggregate in the endoplasmic reticulum and preventing its secretion to the extracellular matrix.[26] The absence of protein does not cause disease, however, intracellular accumulation of myocilin aggregates may sensitize trabecular meshwork cells to apoptosis.[27]

The second gene optineurin (*OPTN*) was identified at *GLC1E* (10p15-p14) and is primarily responsible for rare cases of familial normal tension glaucoma.[28-30] Optineurin may possibly influence ganglion cell apoptosis directly through rab8 signaling.[31,32]

WD repeat domain 36 (*WDR36*) at *GLC1G* (5q21.3-q22.1) seems to be related to POAG severity in some cases, although it is neither necessary nor sufficient for disease development.[33,34] A zebrafish homolog of *WDR36* stimulates apoptosis mediated by p53, implying a possible role for the gene in retinal ganglion cell susceptibility to apoptotic cell death.[35]

Genome-wide scans using nonparametric linkage methods in different populations of POAG pedigrees identified 10 genomic regions that may harbor POAG susceptibility genes (2p14, 2q33-34, 10-12-p13, 14q11-q22, 17p13, 17q25, 19q12-q14).[36-38] Using ordered subset analysis with the mean family age of onset as a covariate, a follow-up study of the scan performed on European descent pedigrees, distinguished some families with significant linkage to 15q11- q13, designated *GLC1I*.[39]

TANK-binding kinase 1 is an enzyme encoded by *TBK1* that can mediate NFKB activation in response to certain growth factors. The gene is specifically expressed in retinal ganglion cells affected by glaucoma. Located in chromosome 12q14, duplications of the gene were discovered in normal tension glaucoma patients. This duplication leads to increased transcription of *TBK1*.[40,41] Besides, *TBK1* associates with the product of *OPTN*.[42] Nevertheless, this is a rare observation, given that, only 1% of patients displayed duplication of the TBK1 gene in a multicenter case-control study.[43]

Useful genetic screening tests for POAG are not available.[44] Currently only 30% of individuals at risk for early-onset forms of glaucoma cases can be identified.[45] Continuing research using genome-wide association in large population may reveal new genetic biomarkers and useful screening tests.[46]

In order to use genes as biomarkers, one needs to have causative genes or to have genes that are associated with disease. At present, gene-based risk prediction and prognosis at early stages of the disease are possible; however, studies that isolate genes associated with late onset forms of glaucoma are still underway. Most of the genes associated with glaucoma are causative, so that, a molecular diagnosis and genetic counseling with families who carry disease are possible. Future research will aim to target newly identified genes with clinical phenotypes and outcomes, to identify genes associated with POAG, and to correlate genetic variation with disease, clinical outcome, and treatment response.

Proteomic biomarkers

The term *proteomics* was first introduced in 1994 for the aim of global characterization of a proteome (referring the proteins expressed by the genome), including protein expression, structure, modifications, functions, and interactions.[47] The proteome is the entire set of proteins, produced or modified by an organism or system.[48]

Proteomics is one of the most important post-genomic approaches to improve the understanding of gene function. Nevertheless, when compared to genome, proteome is a much more complex and dynamic system. Although proteins provide the most important clues to disease mechanisms, their analysis is difficult due to large diversity in properties, such as molecular size, dynamic range in quantity, and their hydrophilicity or hydrophobicity.[49] Conversely, since blood samples can be easily collected, the proteins detectable in serum or plasma have formed the basis of commonly used tests to screen and monitor disease biomarkers in various fields.

Proteomics is highly useful in the identification of candidate biomarkers (proteins in body fluids that are of value for diagnosis), identification of the bacterial antigens that are targeted by the immune response, and identification of possible immunohistochemistry markers of infectious or neoplastic diseases.[50] Recent studies of glaucoma using proteomics analysis techniques have resulted in a lists of differentially expressed proteins in human glaucoma and animal models. The global analysis of protein expression in glaucoma has been followed by cell-specific proteome analysis of both retinal ganglion cells and astrocytes. The proteomics data have also guided targeted studies to identify post-translational modifications and protein-protein interactions during glaucomatous neurodegeneration. In addition, recent applications of proteomics have provided a number of potential biomarker candidates.[49]

To date, most of the studies in glaucoma molecular biomarkers comprise the studies of autoantibodies and their target antigens. A panel of antigenic proteins that elicit serum immunoreactivity at a high frequency among glaucoma patients can provide an effective tool for biomarker screening.[49] However, a much lower abundance of most protein biomarkers than some disease-irrelevant serum proteins poses a challenge of serum biomarker detection.

Currently, 22 proteins were detected in glaucoma patients and included immune mediators and components of cell death signaling which may serve as biomarker candidates (Table 1).[49] Nevertheless, the clinical validation of candidate molecules still poses a major challenge. Large studies of heterogeneous cohorts for appropriate statistical power and blinding are deemed necessary to eliminate false positives and to calculate the sensitivity and specificity of candidate molecules for clinical prediction.[51,52] Besides, given the highly complex pathogenesis and the characteristic inter-patient heterogeneity of glaucoma, a panel of biomarkers, rather than a single biomarker, is needed to provide appropriate sensitivity and specificity needed.[49]

Table 1. Potencial glaucoma protein biomarkers candidates (adapted from Tezel[49]).

Protein name	Accession number*
A-kinase anchor protein 10, mitochondrial precursor	gi|21493033
Actin, cytoplasmic	gi|45011885
Heterogenius nuclear ribonucleoprotein C-like	gi|282396082
Insulin-like growth factor 2 mRNA-binding protein 2 isoform b	gi|56118219
Rho guanine nucleotide exchange factor 40	gi|50843837
Toll-like receptor 8 precursor	gi|20302168
Tripartite motif-containing protein 5 isoform delta	gi|203046698
RNA polymerase I-specific transcription initiation factor RRN3	gi|93102377
Minichromosome maintenance complex component-like isoform a	gi|209954821
Hypothetical protein LOC100510472	gi|310133112
GRIP and coiled-coil domain-containing protein 2	gi|31563507
DNAJ homolog subfamily C member 7 isoform 2	gi|221219056
Zinc finger protein 804B	gi|31791053
1-phosphatidylinositol-4,5-biphsophate phosphodiesterase gamma-1 isoform b	gi|33598946
C-Jun-amino-terminal kinase-interacting protein 1	gi|4885433
Kinesin-like protein KIF17 isoform a	gi|170784807
NACTH, LRR and PYD domains-containing protein 6	gi|21264320
Sialic acid-binding Ig-like lectin 5 precursor	gi|4502659
Testis-specific serine/ threonine-protein kinase 2	gi|194294513
Poly [ADP-ribose] polymerase 1	gi|156523968
NACTH, LRR and PYD domains-containing protein 8	gi|33667040
Protocadherin gamma-A11 isoform 1 precursor	gi|11128039

* GenInfo Identifier (gi) was an early system used in bioinformatics to access GenBank and related databases. A gi number was assigned to each nucleotide and protein sequence accessible through the NCBI search systems, and was a means of tracking changes to the sequence. It is an unique identifier given to a DNA or protein sequence record to allow for tracking of different versions of that sequence record and the associated sequence over time in a single data repository.

Autoimmune biomarkers

There is growing evidence implying an autoimmune involvement in the pathogenesis of glaucoma. A number of studies provide fundamental insights into neurodegenerative properties of autoreactive IgG antibodies, which impair retinal ganglion cells (RGC) survival by specific binding, and assume direct and indirect triggered pathways for cell death *in vivo*.[53]

Additional evidence of the role of autoimmunity in glaucoma is provided by the finding of elevated levels of antibodies against small heat shock proteins (a-A-crystalline, a-Bcrystalline, and HSP27) in normal tension glaucoma patients.[54,55] Disease-specific changes in complex profiles of naturally occurring IgG autoantibodies were detected in the sera of glaucoma patients.[56-58] Increased antibody levels (e.g. HSP70, anti-phosphatidylserine, g-enolase, glycosaminoglycans, neuron specific enolase, glutathione-S-transferase, a-fodrin, vimentin, MBP, glial fibrillary acidic protein, and retinal S-antigen) were identified and implicated as player for autoimmunity in glaucoma and also significant and selective downregulations (e.g. anti-GFAP, anti-14-3-3) could be detected in glaucoma patients.[59-68] However, whether the autoantibodies have a causative effect or appear as an epiphenomenon of the disease is yet to be unraveled. The downregulations are possibly related to a loss of natural protective autoimmunity and a disbalance of naturally occurring autoantibodies promoting neurodegenerative processes.[69,70] This unsteadiness may shift the physiological balance of protective immunity into a neuroinflammatory degenerative process leading to a predisposition for glaucoma which raises the question whether elicited autoimmunity can cause RGC loss.[53]

There is a controversial debate whether autoantibodies are aberrant and contribute to disease pathogenesis or are beneficial, being part of a protective mechanism. Contradicting the principle that autoantibodies are always associated with pathological conditions, cumulative evidence demonstrate that natural autoantibodies entail protective characteristics and that autoimmunity can be protective in some situations.[71,72] Accordingly, the downregulation of some autoantibodies in glaucoma patients could lead to a loss of protective autoimmunity.[53]

As a parameter associated with the presence and severity of specific disease states, autoantibody patterns are useful biomarkers for glaucoma diagnosis before its clinical manifestations. Using mass spectrometry-based proteomics to compare the autoantibody profiles in body fluids (serum, aqueous humor or tears) from patients with glaucoma with those obtained from healthy individuals, autoantibody patterns that are the most discriminating can be classified.[53] Autoantibody profiles are useful laboratory markers for the diagnosis of diseases such as cancer, rheumatoid arthritis and Alzheimer's disease.[73-75] In glaucoma, the complex antibody profiles are stable and consistently exist among different study populations.[65] As described previously, many autoantibody reactivities are significantly increased or decreased in glaucoma patients as compared to non-glaucoma control group. Using a pattern recognition algorithm such as artificial neural networks for unique serum autoantibody patterns, it is possible to differentiate between sera of POAG patients and healthy subjects with a sensitivity and specificity of approximately 93%.[53] Hence, autoantibodies can be highly-specific and accurate useful biomarkers for glaucoma diagnosis by simple blood testing.

Miscellaneous

Some non-genetic molecular candidate biomarkers includes hormones such as erythropoietin, which exert its neuroprotective effect by reducing the nitric oxide-mediated formation of free radicals or antagonizing their toxicity, and hepcidin that regulates of iron efflux from numerous cell types and is expressed in the Müller cells, photoreceptors, and retinal pigmented epithelium. Table 2 depicts a list of potential non-genetic glaucoma biomarkers.[76]

New proteins detected in the aqueous humor of glaucoma patients are involved in molecular events that resemble those that occur during atherosclerosis, such as, endothelial dysfunction, lipoprotein alteration, modification of smooth muscle cell functions, oxidative damage, inflammation, loss of intercellular adhesion, mitochondrial failure, and apoptosis.[77] As a whole, these observations indicate that a remarkable endothelial damage affects the anterior chamber in glaucoma, especially in the trabecular meshwork. From a biological point of view, the anterior chamber is a space that is surrounded by an endothelium and a path by which a liquid travels, so it can be considered as being similar to a vessel.[78] Hence, these new proteins are referred as vascular biomarkers (Table 2).

Neurodegenerative markers

Neurodegenerative diseases are slowly progressive and irreversible disorders of the nervous system. Early detection of disease is possible by means of neurochemical measurements and neuroimaging biomarkers specifically related to the pathogenic events.[79,80] Parkinson's and Alzheimer's disease are typical neurodegenerative diseases and although its primary causes are different from the glaucomatous optic neuropathy, they share close similarities in several pathological findings.[81]

Retinal ganglion cell bodies are located within the eyeball and its axons emerge the eye forming the optic nerve, chiasm and optic tract. As retinal ganglion cell axons project to the central nervous system, their number within the retrobulbar optic nerve may be a suitable surrogate marker for optic atrophy.[82] Thinning of the retrobulbar optic nerve has been reported both in histological and diagnostic imaging studies suggesting that the diameter of the nerve may correlate with the extent of the optic atrophy.[83-87] High-resolution magnetic resonance imaging (MRI) using an ultra fast HASTE-sequence at 3 T sequences of the optic nerve can portray axonal loss in the optic nerve comparing closely with the retinal nerve fiber layer-related parameters and could be used as a biomarker for axonal loss in glaucoma.[82] 3-T diffusion tensor imaging of the optic nerve in patients with glaucoma displays good correlation with the retinal nerve fiber layer thickness measured by OCT and may serve as a biomarker of disease severity.[88]

The majority of the ganglion cells axons terminate in the lateral geniculate nucleus (LGN), the major relay station between the retina and the visual cortex.[89] In an experimental glaucoma model on monkeys, the loss of optic nerve fibers leads to degenerative changes in the LGN, with decrease in number and size of neurons and overall nucleus shrinkage.[90,91] These findings provide evidence of trans-synaptic degeneration in glaucoma, and may be relevant to understanding disease spread in select patients.[81] *In vivo* MRI evidence of LGN degeneration in human glaucoma is consistent with *ex vivo* primate and human neuropathological studies. LGN atrophy may be a relevant biomarker of visual system injury and/or progression in some cases of moderate to severe glaucoma patients.[92]

Table 2. Non genetic candidate biomarkers in glaucoma (adapted from Kokotas et al[76]).

Candidate biomarker	Type	Source
3 α -HSD	enzyme	blood
Ankyrin-2*	protein	AH
ANGPTL7	protein	TM and AH
Antibody for glycosaminoglycans	antibody	serum
Antibody for GST	antibody	serum
Antibody for NSE	antibody	serum
Antibody for heat shock proteins	antibody	serum
Antibody for anti--Helicobacter pylori	antibody	AH and serum
Antibody for Chlamydia pneumoniae	antibody	serum
AP_4A compound	nucleotide	AH
Apoliprotein B and D*	proteins	AH
BDNF	protein	serum
Caspase-14	enzyme	AH
CD44H	protein	TM and AH
Cellular senescence	antagonistic pleiotropic response	AH
Citrate	multifunctional acid	Plasma
Cystatin C	protein	AH
Cytokines	proteins	TM, AH, and serum
ELAM1*	protein	AH
Erythropoietin	hormone	AH
GRP78	protein	TM
Heat shock 60 and 90 kDa*	proteins	AH
Hepcidin	hormone	AH
Homocysteine	amino acid	AH, plasma, and tear fluid
Hydroxyproline	imino acid	AH and plasma
Malondialdehyde	aldehyde	AH and plasma
Myoblast determination protein 1*	protein	AH
Myocilin	protein	AH and TM
Myogenin*	protein	AH
Myotrophin*	protein	AH
Nitric oxide synthase	enzyme	TM, Schlemm's canal, and collecting channels
PGDS	enzyme	AH, TM, and serum
Phospholipase A_2	enzyme	TM
Phospholipase C β and γ*	enzyme	AH
Transferrin	protein	AH
Transthyretin	protein	AH
Tumor necrosis factor α	protein	AH
Ubiquitin fusion degradation 1-like*	protein	AH
Vasodilator stimulated phosphoprotein*	protein	AH and TM

AH: aqueous humor; TM: trabecular meshwork; * vascular biomarkers[77]

Future developments and conclusion

In summary, a biomarker is an anatomic, physiologic, biochemical, or molecular parameter associated with the presence and severity of specific disease states. A biomarker may be detectable and measurable by a variety of methods, including physical examination, laboratory assays, and medical imaging. As a laboratory measurement or physical sign used in therapeutic trials as a substitute for a clinically meaningful endpoint, surrogate endpoints are used as direct measures of how a patient feels, functions, or survives and are expected to predict the effects of the therapy. For validation, a biomarker has to exhibit the capability to capture the net effects of treatment on clinical outcome, using an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic, or clinical significance of the test results.[5]

The glaucomatous optic neuropathy encompasses a number of different forms of disease, from childhood, early onset juvenile glaucoma to secondary and adult glaucoma. The discovery of specific biomarkers for each particular glaucoma is deemed necessary and poses a challenge to researchers. Hence, there is probably no single 'ideal' glaucoma biomarker that is going to cover all aspects of clinical disease including early detection, severity prediction, progression, and response to treatment.[5]

Despite the plethora of candidates biomarkers discusses in this review, there are still unmet needs for glaucoma. What are the candidate genes that affect connective tissue biomechanics and how would that relate to glaucoma susceptibility? Is there any biomarker that indicates the speed of disease progression? Future research should focus on these issues.

In the near future, as physicians, we expect to be able to establish a patient's risk for POAG using a combination of genetic, clinical and biochemical markers, to assess the ganglion cell disease by novel imaging techniques, and initiate appropriate therapy to restore ganglion cell health.[76]

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