DIAGNOSTIC POTENTIAL OF TEARS IN OPHTHALMOLOGY

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SUMMARY

In research community increases the need for the search and identification of new ways in diseases diagnosis, following-up the progression of the disease, and response to the treatment. The detection technologies sensitivity improved significantly and it makes possible the quantification of detected substances in very small amounts. The tears are biological material with constantly developing possibilities in the diagnosis of different diseases. Our goal was to compile the basic overview of diagnostic potential of the tears using summary of potential tears biomarkers of different diseases. In the paper, there are described proteins biomarkers studied especially in the last years, which correlate with specific eye disease (dry eye, allergy, glaucoma, etc.). It summarizes until now published results from the range of systemic diseases in patients with scleroderma, cystic fibrosis, diabetes mellitus, multiplex sclerosis, tumors, and Parkinson's disease as well. It focuses on proteomic analyses with the goal to specify more effectively markers, which might be used in the early diagnosis of eye diseases in the clinical practice in the future as well.

Key words: tears, biomarkers, eye diseases, systemic diseases

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INTRODUCTION

A biomarker is an indicator of a physical condition which can be measured precisely and reproducibly. On the basis of its measured values, it is possible to confirm or disprove the presence of a pathology. In addition to the fact that it serves as a diagnostic tool, it also finds use in monitoring the genesis and development of a pathology, as well as response to pharmacological treatment and its success rate. It is important for its levels to correlate with the symptoms specific for the given pathology, the clinical manifestations and the standard diagnostic tests. In the search for new appropriate and reliable biomarkers of various pathologies several criteria are assessed, within the framework of which a simple approach to the sample material contributes to a positive assessment of suitability. Sampling of tears is non-invasive, possible and accessible. This physical fluid contains potential biomarkers of both ocular and systemic pathologies.

Tears are a product of the lachrymal gland (glandula lacrimalis) located beneath the upper eyelid and the accessory lachrymal glands (gll. lacrimale accessoriae) located in the conjunctiva. They contain proteins (mucins, enzymes, glycoproteins, immunoglobulins), lipids, electrolytes, water and organic soluble substances. Tears form the lachrymal film on the surface of the eye – a protective layer, which ensures continual lubrication. The lachrymal film consists of a water layer, composed predominantly of proteins, water and electrolytes, whilst the lipid layer contains several types of lipids (for example glycerol esters – triacylglycerols, wax esters, free fatty acids, polar lipids) and neutral diesters (16). Although the volume of the sampled tears is small (~20 µl), the development of techniques enables an analysis of 6-12 mg/ml of proteins in the lachrymal fluid even in these quantities (58). The most frequently used method of sampling tears is microcapillaries. Sampling itself takes place by the attachment of a microcapillary to the temporal edge of the eyelid, without contact with the conjunctiva. If the quantity of tears is not sufficient, sampling of tears is implemented with the aid of rinsing with a physiological solution. The method of rinsing takes place by the application of a drop of solution into the conjunctival sac, after which a tear sample is taken using a micropipette.

The determination of lachrymal proteins is significant for understanding fundamental pathological processes, in timely diagnosis and treatment of various serious pathologies, which include pathologies with a high degree of loss or serious damage to sight (diabetic retinopathy, age-related macular degeneration (ARMD), primary open-angle glaucoma (POAG) etc.), as well as systemic pathologies such as cystic fibrosis, cancer and Parkinson's disease (19). A further advantage is analysis of topically applied pharmaceuticals in ocular pathologies.

This study provides an overview of the most significant lachrymal proteins and factors whose use could contribute to the timely and reliable diagnosis of certain types of serious pathologies. This information could contribute to slowing or halting the progression of pathologies, to quicker and correct diagnosis for the purpose of timely treatment, or targeted suppression of manifestations of pathologies.

Potential of biomarkers of selected ocular pathologies in lachrymal fluid

Dry eye syndrome

Dry eye syndrome is an inflammatory disease affecting the surface of the eye. Its symptoms include burning and stinging in the eyes, fibrous mucus in or around the eyes, sensitivity to light, reddening of the eyes and a feeling of a foreign body in the eyes. It has a high prevalence among the American, Wes-

tern European and Asian population above 50 years of age (20), which sometimes reaches up to 50% (43). The etiology of the syndrome is very diverse. The main cause is considered to be a deficit of the water layer of the lachrymal film or changes in the lipid component. A typical clinical manifestation is increased levels of osmolarity and inflammatory cytokines in the patient's tears (26), (35). Since inflammation is a key component of dry eye syndrome, in seeking a biomarker for this pathology contemporary studies focus especially on an analysis of cytokine molecules. Further research categories of potential markers are growth factors, mucins, neuromediators and lipids (18).

A change of protein expression takes place during the pathology. Upon dry eye syndrome significantly higher concentrations of IL- (interleukin) 1, 6, 8, 17, 22, TNF-α (tumour necrosis factor α) have been detected, which may further intensify inflammation by activating immune T-cells (27), (19). Further studies on the suitability of their use as markers of this pathology are necessary due to the lack of specificity, since their levels increase in the majority of inflammatory reactions. A further observed inflammatory marker is IFN-□ (interferon □). This leads to a loss of cup cells as a result of increased apoptosis of the corneal epithelium and an increased formation of matrix metalloproteinases (27), which leads to a further progression and worsening of the symptoms of the pathology. The aforementioned matrix metalloproteinases are determined in contemporary diagnostic practice with the use of freely available tests for MMP-9 (matrix metalloproteinase 9), which serves as the current biomarker of this pathology, although it is not specific thereto. A number of studies have confirmed that markedly increased values upon dry eye syndrome are manifested also by pro-inflammatory proteins from the group S100, specifically S100A8 and S100A9 (11, 32, 54), indicating their engagement in the inflammatory response. In the studies published to date, of the aforementioned potential inflammatory biomarkers, none have been found that would fulfil the conditions of high specificity, correlation of its surface with the progression of the pathology and simultaneously sufficiency in the reflection of the influence of exogenous factors. The lack of a correlation between the symptoms and manifestations in changes of the levels of the selected biomarker substantially complicates diagnosis, and causes frequent failure of clinical attempts to seek and introduce a relevant biomarker as a consequence of the lack of a reliable evaluation.

Of the proteins not directly contributing to the inflammatory response, studies indicate the usability of lysozyme, one of the most recently studied proteins in tears. It is not specific to dry eye syndrome, but may be used in this pathology e.g. as a control for the adverse effects of pharmaceuticals such as blockers of β-adrenergic receptors (11).

Dry eye syndrome associated with graft versus host disease

A high degree of similarity with dry eye syndrome is manifested by Graft versus host disease (GVHD) – associated dry eye, a chronic form of ocular pathology. This appears in 30 – 70% of patients who undergo treatment of allergenic transplantation of haematogenous stem cells (5). As in the case of dry eye syndrome, the patient may feel stinging and burning in the eye, reddening or a feeling of a foreign body in the eye. Research groups concentrate on various inflammatory cytokines in tears, and further studies are required in order to confirm or disprove their suitability. Other possibilities include the construction of a panel of several biomarkers which would increase diagnostic and prognostic strength.

Of the 84 investigated genes in patients with GVHD, 34 manifested significant changes in comparison with the control group (5). IL-6, IL-9, CCL-24 (C-C motif chemokine ligand 24), CCL-18, IL-10, IFN-γ and CCL-2 were increased by more than six times in the epithelial cells of the conjunctiva. However, sampling of lachrymal fluid is less invasive than sampling the conjunctival epithelium, and as a result suitable biomarkers are sought in this bodily fluid. Of the selected cytokines, good sensitivity and specificity were manifested by IL-8 and interferon-inducible protein 10, although the number of these studies is small and confirmation of the results requires a prospective model of a study within a larger population (5).

Sjögren's syndrome

Sjögren's syndrome is a sub-group of dry eye syndrome with a deficit of the water layer of the lachrymal film. This concerns an autoimmune disorder, in which the endocrine glands are affected, specifically the lachrymal, salivary, sweat and mucous glands, as well as the glands of the pancreas, small intestine and respiratory tracts. Two of the most common symptoms are xerophthalmia and xerostomia. The pathology particularly afflicts women aged between 40 and 50 years (41). The etiology of the pathology is not yet fully understood or clarified. The results of studies in this area indicate that in sensitive individuals a self-triggering inflammatory response develops through the influence of exo- or endogenous antigens.

With regard to the various manifestations of the pathology in individual cases and the similarity with other morbidities, diagnosis remains difficult, and the need for a suitable biomarker remains a pressing issue. Biochemical examinations confirm increased values of cytokines IL-1α, IL-1β, IL-6, IL-8, TNF-α and MMP-9 (56). They also detected increased quantities of lachrymal autoantibodies against Ro/SSA, La/SSB and IgG (immunoglobulin G) together with IgA (immunoglobulin A) against α-fodrin, the use of which continues to be the focus of study. AQP5 (aquaporin 5) is another protein whose levels are increased in tears upon Sjögren's syndrome, but not in dry eye syndrome (56), indicating that AQP5 could penetrate into tears upon the destruction of the lachrymal gland by lymphocytes. This hypothesis is supported also by the detection of serum antibodies against AQP5, which were significantly higher in patients with Sjögren's syndrome, increasing its chances as a future biomarker of this pathology (1).

Age-related macular degeneration

Age-related macular degeneration (ARMD) is a commonly occurring pathology and a cause of loss of sight in persons aged over 60 years, during which a progressive degeneration of retinal cells in the central part takes place in various phases, with a change in the composition of its proteins. The progression of the disorder various among patients. In some cases the development of ARMD may proceed slowly, without eventual loss of sight, but in the case of a more rapid course it

results in a loss of vision in one or both eyes. A basic symptom is the appearance of blurred spots close to the centre of the visual field, which increase in size over the course of time and may grow into blind spots . Risk factors include smoking, race and genetic predisposition. The early to medium stages are usually asymptomatic, in these stages it is possible to detect the pathology by means of a complex eye test. At present the most commonly used therapy for ARMD is injection of anti -VEGF (vascular endothelial growth factor) antibodies, which block the abnormal growth of blood vessels (22). However, anti-VEGF therapy is not universal, and in fact worsens progression in the case of non-neovascular ARMD (14).

A proteomic analysis of a neurosensory retina from the macula detected a change in the proteins which contribute to the regeneration of proteins of the mitochondria of the retinal pigment epithelium. There was a reduction of the level of proteins in the neurosensory retina, which contribute to the formation of microtubules, damage occurred to the cytoskeleton and to the transport of proteins between the inner and outer segment. The result was a loss of plasticity of the retina and its regenerative capacities. On the basis of these findings, the possibility is being outlined of investigating the suitability and expediency of biomarkers of the type of ARMD, tubulin and cofactor A, which point to damage of the formation of microtubules. Potential is also shown by stathmin, demonstrating a loss of plasticity in the retina, and chaperone Hsp-60, which provides information about the repackaging of proteins (3). Various proteins have been discovered with an increased presence in the tears of patients with ARMD, but especially albumin and serotransferrin (33). Tear proteome lags behind in this pathology in comparison with other ocular pathologies, and a number of potential biomarkers of ARMD have been detected in other biological fluids (18, 28). A non -invasive biomarker from tears would be an immense contribution for timely diagnosis and understanding of the etiology and progression of the pathology. This area has not been investigated and offers broad possibilities for research studies.

Allergic conjunctivitis

The term allergic conjunctivitis covers a set of allergic pathologies affecting the eyelid and conjunctiva. It is manifested in lachrymation, reddening of the skin in the surrounding area of the eye, itching and loss of eyelashes. The prevalence of any of the forms of ocular allergies is one half of the world's population (30). Manifestation of the pathology occurs following contact with the relevant allergen (dust, pollen, animals...).

Diagnosis of the initiation phase or chronic stages does not represent a problem, but it is difficult to differentiate the pseudo-allergic forms with similar clinical manifestations from other pathogenesis of the disease, e.g. acute and chronic infection, blepharitis, dysfunction of the lachrymal film etc. Distinguishing an allergic reaction is helped by information about the presence of increased serum specific IgE, but the result may not correlate with an ocular pathology. Increasing specificity enables a determination of the specific lachrymal IgE against a specific allergen, which provides information about local sensitivity toward the given allergen (31). IgE rank among mediators of allergic ocular reactions together with histamine, tryptase, chymase, ECP (eosinophil cationic protein), IL-4 and several other proteins (30). Their potential use as biomarkers is complicated in particular by the lack of specificity for allergic reactions. However, as in the case of chymase, which reflected the severity of conjunctivitis (12), they may be of use in assessing the stage of the pathology.

Trachoma

Trachoma is an infectious disease commonly occurring in developing regions such as Africa, the Middle East and Asia, causing blindness. The infectious agent is Chlamydia trachomatis, which causes trichiasis, resulting in repeated scarring of the cornea and conjunctiva. It is estimated to affect 21 million people, of whom 2.2 are blind and 7.3 million of whom have trichiasis (53). It spreads upon direct contact with the eye, nose and secretions of the throat of the affected individuals, or through contact with contaminated clothing.

In diagnostics the standard procedure is to send a sample of bacteria from the eye for a laboratory test, but in the places of its incidence these tests are often unavailable. Control or elimination of the pathology requires a reliable testing method. Research has been under way for a long time in the area of detecting immune responses to trachoma by means of immunoglobulin in tears. Patients with the inflammatory type of trachoma have manifested significantly higher IgG against antigens of chlamydia cHSP60 (chlamydia trachomatis heat shock protein 60), protein CT795 and CPAF (chlamydial proteasome/protease-like activity factor) (19). The search is continuing also between IgM and IgA, but is also gradually developing for further protein molecules. With the aim of improving knowledge in the pathology of trachoma, the expression of selected cytokines in tears was monitored. Levels of EGF (epidermal growth factor), TGF-β1 (transforming growth factor β1) and TNF-α were linked with the formation of conjunctival scars and have the potential to serve as markers of the progression of the pathology (49).

Keratokonus

Keratokonus is a progressive thinning of the cornea, an ectatic state which influences vision. Its initial manifestations include deterioration of visual acuity, in the later stages an increase in myopia and astigmatism. It occurs in 1 out of 500 to 1 out of 2000 individuals, in which it has a higher prevalence among the Asian population (38). The etiology and pathogenesis is not entirely clarified. Factors contributing to the development of the pathology may be of genetic origin and also from environment and the presence of ocular allergies, Down's syndrome, tapetoretinal degeneration, and atopy are also linked with keratoconus. Although this pathology is bilateral, the clinical and topographical changes are often present in only one eye.

Biochemical examinations of tears demonstrate a very similar increase in the level of cytokines, MMP and a reduction of lipocalin, lactoferrin and lysozyme as in the case of patients with dry eye syndrome (38), which thus excludes these biomarkers from selection due to their non-specificity. In the case of diagnosis of dry eye syndrome, a correlation has also been confirmed with the level of AZGP1 (zinc-α2-glycoprotein) (55), the diagnostic use of which is being investigated in the case of keratoconus (38, 19). AZGP1 bonds to PIP (prolac-

tin-inducible protein), which although it is being investigated especially in connection with breast cancer, its regulation was significantly altered in the tears of patients with keratoconus (44), attributing high potential to this protein as a biomarker of the pathology in question. Among several other potential biomarkers are SFRP1 (secreted-frizzled related protein 1), Ig κ, ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1), which require further studies to confirm or disprove their effect (38).

Glaucoma

Glaucoma is a pathology affecting the internal structure of the eye, in which there is a blockage of the drainage of chamber fluid, causing ocular hypertension. The prevalence is approximately 2% in people aged over 40, and is higher in the Afro-Caribbean population (19). The pathology still remains the primary cause of irreversible damage to sight. Four different forms are usually distinguished: primary open -angle glaucoma (POAG), primary closed-angle glaucoma, secondary glaucoma as a consequence of another pathology, and congenital glaucoma. The diagnosis is based on an assessment of intraocular pressure, the visual field and papilla of the optic nerve, in acute cases on the basis of symptoms such as hard red eye with the pupil not reacting to light, disorders of vision and pain. The course of POAG is asymptomatic for a long time, which increases the need for a suitable biomarker, thanks to which it would be possible to detect the pathology in a timely manner. Identification of specific molecular markers for glaucoma could detect the early phases of the pathology, predict the prognosis and monitor the effectiveness of therapy.

Several potential biomarkers of the pathology are in the stage of verification of their suitability and usability. In patients with glaucoma there is an alteration of the regulation of inflammatory proteins, which may further be linked to the mechanism by which prostaglandins reduce intraocular pressure. An increased expression of the inflammatory cytokines IL-1β, IL-6, IL-12 and TNF-α (34) was observed in the tears of patients with POAG who underwent topical therapy. In contrast with this study, Chong et al., upon an investigation of cytokines in patients with glaucoma using medicamentous therapy, found a significant increase only in MCP-1 (monocyte attractant protein 1) (4). Further studies in the given area are necessary for clarification of the results. BDNF (brain-derived neurotrophic factor) is a further potential biomarker, which was fundamentally reduced in patients with normotensive glaucoma (19), and in 2010 was registered in the database of patents. However, it has not yet found application in clinical practice.

Keratopathy

The term keratopathy is used in order to designate any dysfunction or pathology of the cornea. In connection with the search for biomarkers, several study have focused on "climatic droplet keratopathy" (CDK), a pathology characterised by the progression of turbidity of the anterior layer of the cornea. Studies have detected significantly increased values of N-glycosylated haptoglobin in the tears of patients with CDK, which is linked to exposure to UV radiation, and this could contribute to a better understanding of the etiology or the discovery of a biomarker (50). Other lachrymal biomarkers of CDK which are the focus of examination include MMP-2 and MMP-9 (21). They have also been examined in connection with another form – diabetic keratopathy, where they are probably responsible for local restructuring of the tissue and progression of the pathology. Due to these mechanisms they could serve as timely biomarkers of the pathology (19), and their inhibitors as prevention of progression of the pathology (50).

Aniridia

Aniridia is a rare hereditary disorder, in which the iris is either incomplete or entirely lacking. It usually afflicts both eyes. With regard to the influence of heredity in this pathology, research concentrates on the relevant genes, and as a result there are only very few studies engaging with lachrymal preteomics in these patients. A proteomic analysis of tears of patients with aniridia identified a number of proteins, the level of which was markedly altered. Five proteins manifested low values: α-enolase, peroxiredoxin 6, cystatin S, gelsolin and apolipoprotein A-1, three proteins manifested increased values: AZGP1, lactoferrin and VEGF-C. Their use shall be the subject of future studies (23). Diadenosine polyphosphates also rank among potential markers which have been investigated in connection with dry eye syndrome, but their levels were significantly increased also in patients with aniridia (42). Further research could confirm or disprove their correlation with the progression of corneal defects in this pathology.

Wearing contact lenses

Upon wearing contact lenses, changes occur in the protein profile, as a consequence of which a disruption of the protective nature and dynamics of the lachrymal film may occur (56). Long-term wearing of contact lenses is often accompanied by side effects such as the development of dry eye syndrome, problems with the cornea such as edema or blurred vision as a consequence of hypoxia, allergic reaction, inflammation in the eye or eyelid (10). Research of proteomics in this area is essential for the development of contact lenses and an understanding of the metabolic processes which are connected with regular wearing.

In the group which wore soft contact lenses, increased values of S100A8 were measured, as well as a decrease in the values of lysozyme, whereas in the group with rigid gas permeable hard lenses cystatin was increased and secretoglobin decreased (56). The expression of twelve pro-inflammatory cytokines in tears of contact lens users was significantly higher, but this was demonstrated without a correlation to the feeling of comfort in the eyes when wearing contact lenses (57). Changes in the levels of cytokines were identical to the measurement of hyperaemia and dryness (24), and changes to their levels occurred also in the case of lenses designated for 30-day wearing (25).

Systemic pathologies with ocular complications

Diabetic retinopathy

Diabetic retinopathy (DR) ranks among chronic complica-

tions of diabetes mellitus, which may cause loss of sight and other serious metabolic, vascular and neurological damage in patients in productive age. Since timely diagnosis may significantly slow the progression of the pathology, patients undergo screening with the use of ophthalmoscopy or ever increasingly popular photographic methods (9). The aforementioned procedures are not without unpleasant side effects, such as flashes in vision, which may persist for a further 3-5 minutes. A more comfortable and less demanding approach would be enabled by a suitable biomarker. Research is oriented towards various inflammatory factors, growth factors and angiogenic factors, which contribute to the activation of the endothelium, leading to dysfunction of blood vessels. The capacities of lachrymal biomarkers to reflect changes in the retina remains questionable, since tears are not in direct contact with the retina. However, upon DR vascular changes take place, which lead to changes in the retina and therefore the altered flow of blood may have an impact on a change to the composition of tears secreted from the lachrymal gland (9). In connection with this pathology a number of proteins have been the subject of investigation, but as yet there are no tissue-characteristic biomarkers that would be specific only to the retina. Under study among biomarkers for DR, due to altered angiogenesis, are VEGF (39) and IL-6 (interleukin 6) (15), IP-10 (interferon-induced protein 10), MCP-1 (monocyte chemoattractant protein 1) (56), but also several others to a lesser degree. The presence of hyperglycaemia is a trigger of several metabolisms of the signal pathways, which lead to a change in the levels of IGF (insulin-like growth factor) (45). Further intensively studied lachrymal proteins in connection with DR are NGF (nerve growth factor), APOA1 (apolipoprotein A1), lipocalin 1, lactotransferrin, lacritin, lysozyme C, lipophilin A, λ chain of immunoglobins, Hsp27 (heat shock protein 27), β2-microglobulin and enolase (9). As yet it is not known whether significant changes in the levels of these proteins are a consequence of the pathogenesis of DR or diabetes mellitus.

Diabetes mellitus is accompanied by a restructuring of vascular structures, which is connected with matrix metalloproteinase. Diabetic patients who have undergone vitrectomy manifest markedly higher values especially of MMP-10, but before surgery the levels of MMP-2, MMP-9 and MMP-10 in tears did not differ pronouncedly from the healthy control group (36). MMP-7 decreases in the vitreous body of diabetic patients, in which its levels did not correlate with the levels in blood serum. MMP-7 activates NGF by fission of proNGF, and additionally levels of NGF reflect the persistence of diabetes mellitus, HbA1c and glycaemia (40). More recent studies recommend observation of the early ration of proNGF/NGF, but it is necessary to confirm this hypothesis with the aid of further studies (37).

Administration of anti-VEGF antibodies has become a partially effective therapeutic procedure. In the first two years its effect has been confirmed on reducing further progression of loss of sight and on improvement of vision, but long-term application may cause hypertension as well as other renal side effects – proteinuria, and also glomerular thrombotic microangiopathy (8).

Scleroderma

Scleroderma, also referred to as systemic sclerosis, is a rare disorder affecting the conjunctival tissue, and is characterised by progressive sclerosis of soft tissues (thickening of skin by means of an accumulation of collagen, vasculitis). It is clinically manifested in an affliction of the skin, joints, intestines and other organs. Although the precise etiology is unknown, the central processes in the pathogenesis of the disease are activation of immune processes, vasculopathy and fibrosis (52). Its manifestation in the eye has a high degree of heterogeneity and may be manifested as dry eye syndrome as a consequence of fibrosis of the lachrymal gland, telangiectasia of the conjunctiva, filamentary keratitis (19), changes to the skin of the eyelid, keratoconjunctivitis sicca, cataract and glaucoma (17).

With regard to the generally low incidence within the population, there are only a small number of studies that focus on proteomics in this pathology, and they are frequently limited by their small sample. One of these is an observation of changes in levels of VEGF in tears, which were compared between patients and healthy individuals with regard to the vasculitis-related character of the pathology. VEGF did not manifest any fundamental differences in the investigated population, indicating its unsuitability as a biomarker (46). A potentially more suitable biomarker could potentially be found among cytokines. The precondition of an altered profile of pro-inflammatory cytokines in tears was confirmed, and on the basis of significant changes in levels and molecular characteristics, suitable candidates appeared to be IP-10 and MCP-1 (47). Further research and understanding of the signal pathways of these substances could help in selecting appropriate treatment.

Cystic fibrosis

Cystic fibrosis (CF) is a serious genetic disorder affecting the exocrine glands, with the production of abnormally thick secretions, leading to the dysfunction of organs for which mucus is an important part of their functioning, such as the lungs, intestines, pancreas and sweat glands. In men it is the cause of infertility. It is a life-threatening condition, which due to the accumulation of mucus causes infections in the lungs and digestive complaints in the digestive tract. With regard to its affliction of the epithelium of secreting glands, dry eye syndrome is one of a number of ocular manifestations of the pathology.

The small number of studies dealing with lachrymal proteomics in connection with CF focus mainly on the determination of cytokines. In order to assess the condition of health of the ocular surface in patients IL-8 and IFN-γ were monitored, in which the levels of both were significantly increased in comparison with healthy control subjects. Furthermore, the levels of these two cytokines correlated significantly with the clinical development of CF, indicating an important role of these cytokines in the progression of inflammation of the ocular surface and the pathogenesis of CF (19). A disadvantage of their use is their low specificity, since IFN-γ is increased also in Sjögren's syndrome and dry eye syndrome itself (11), as well as in other inflammatory conditions of the ocular surface. Another potential marker is MIP-1β (macrophage inflammatory protein 1β), which was significantly increased in the tears of patients with CF and dry eye syndrome, although its levels in serum manifested virtually no difference in comparison with control indivi-

duals. Little is known about the role of this protein in the progression and pathogenesis of the disorder, but further research would shed new light and improve our awareness about its usability, either as a biomarker or as a therapeutic target (19).

Systemic disorders without ocular complications

Cancers

Cancers represent more than one quarter of the cause of all deaths within OECD (Organisation for Economic Co-operation and Development) countries, and are the second most common cause of death, after disorders of the circulatory system. A large problem faced by doctors in the case of cancers is late diagnosis. The search for biomarkers is ongoing, in virtually all the bodily fluids, including tears.

Instead of one biomarker, Lebrecht recommends assembling a panel of biomarkers, on the basis of which he succeeded in diagnosing breast tumours (29) with 70% sensitivity and specificity. With regard to the high degree of false positivity in traditional mammograms, the study was highly successful, and research is being applied to the development of a device which would enable accessible, easy and quick diagnosis.

Lacryglobin, a protein secreted by tears and the tear glands, mammary glands and uterus, was found in increased quantities in the lachrymal fluid in patients with various types of tumours. However, it was also present in the tears of control individuals who had a relative within the family with a diagnosis of cancer (13). With regard to the small sample, further research is necessary in order to determine the usability of this potential biomarker.

A lachrymal biomarker or a panel of biomarkers has great potential in the timely diagnosis of cancers, but there are still insufficient studies and research groups focusing on this issue.

Multiple sclerosis

Multiple sclerosis (MS) is one of the most common neurological disorders with an incidence among young adults aged under 30 years. It is an autoimmune disorder in which a demyelinisation of the central nervous system takes place, resulting in problems with balance and co-ordination, muscle weakness, tingling or numbness in the limbs, depending on the place of inflammation, and damage to tissues in the individual parts of the brain or spinal cord. At present diagnosis is performed from the cerebrospinal fluid by means of lumbar puncture, which is a highly invasive method with a large degree of discomfort for the patient. Oligoclonal IgG bands are a standard determined biomarker.

Determining oligoclonal IgG bands is also possible in the lachrymal fluid as a non-invasive alternative to the cerebrospinal fluid. However, the results of such studies are ambiguous or contradictory, and therefore the conclusions concerning the usability and suitability of oligoclonal antibodies as a biomarker from tears are disputable (19).

Upon a proteomic analysis of tears of patients with MS, significantly different values were recorded by α1-antichymotrypsin, with excellent reproducibility of results (48). The protein is the subject of studies of several other pathologies, in which changes of its levels in the serum or plasma of patients are observed, thus its specificity is problematic, and requires further verification.

Parkinson's disease

Parkinson's disease is a progressive neurodegenerative disorder of the central nervous system, occurring in approximately 1.2 million people in Europe (19). The importance of research is increasing also with regard to the change in the age structure of the population. The WHO (World Health Organisation) envisages that in future neurodegenerative disorders will overtake cardiovascular diseases as a cause of death. Incorrect diagnosis occurs in 1 out of 10 patients (2). Diagnosis consists of an evaluation of the clinical manifestations in combination with other additional tests. As a result of its mild phenotypic manifestation in the early stages and the similarity of its symptoms to other degenerative and non-degenerative disorders, a substantial endeavour is under way to find a suitable biomarker for Parkinson's disease.

As in the case of other systemic pathologies, research into lachrymal proteomics in the search for a potential biomarker is in its infancy. An important molecule in the pathogenesis of Parkinson's disease is TNF-α, which causes progressive degeneration in dopaminergic neurones. Its levels in the tears of patients in various stages of Parkinson's disease were significantly higher than in healthy individuals, but were not connected to the duration or severity of the stage (7). At present further studies are ongoing into lachrymal proteomics in patients with Parkinson's disease, and it shall be interesting to follow their impact on the diagnosis of this pathology (59).

CONCLUSION

Scientific and clinical research into non-invasive methods in the diagnosis of various types of pathologies is increasing, which makes lachrymal fluid an attractive sampling material. This information provides an overview of potential protein biomarkers form tears and forms the basis for studies, the aim of which should be to determine and clarify the results of changes to the content of specific lachrymal proteins. The further development of this area could contribute to an innovative approach to diagnosis of the patient, with the aim of personalised and effective therapy.

LITERATURE

1. **Alam, J., Koh, J.H., Kim, N. et al.:** Detection of antibodies against aquaporin-5 in the sera of patients with primary Sjögren´s syndrome. Immunol Res, 64; 2016: 848–856.
2. **Börger, M., Funke, S., Bähr, M. et al.:** Biomarker sources for Parkinson‘s disease: Time to shed tears? Basal Ganglia 5; 2015: 63–69.
3. **Cheryl, M.E., Reilly, C., Feng, X. et al.:** The Proteome of Central and Peripheral Retina with Progression of Age-Related Macular Degeneration. Invest Ophthalmol Vis Sci, 47; 2006: 2280–2290.
4. **Chong, R.S., Jiang, Y.Z., Boey, P.Y. et**

al.: Tear cytokine profile in medicated glaucoma patients: effect of monocyte chemoattractant protein 1 on early posttrabeculectomy outcome. Ophthalmology, 117; 2010: 2353–2358.
5. **Cocho, L., Fernandéz, I., Calonge, M. et al.:** Biomarkers in Ocular Chronic Graft Versus Host Disease: Tear Cytokine- and Chemokine-Based Predictive Model. Invest Ophtalmol Vis Sci, 57; 2016: 746–758.
6. **Cocho, L., Fernández, I., Calonge, M. et al.:** Gene Expression-Based Predictive Models of Graft Versus Host Disease -Associated Dry Eye. Invest Ophtalmol Vis Sci, 56; 2015: 4570–4581.
7. **Comoğlu, S.S., Güven, H., Acar, M. et al.:** Tear levels of tumor necrosis factor -alpha in patients with Parkinson's disease. Neurosci Lett 553; 2013: 63–67.
8. **Coucha, M., Elshaer, S.L., Eldahshan, W.S. et al.:** Molecular Mechanisms of Diabetic Retinopathy: Potential Therapeutic Targets. Middle East Afr J Opthalmol 22; 2015: 135–144.
9. **Csősz, É., Deák, E., Kalló, G. et al.:** Diabetic retinopathy: Proteomic approaches to help the differential diagnosis and to understand the underlying molecular mechanisms. J Proteom 150; 2017: 351–358.
10. **Dillehay, S.M.:** Does the Level of Available Oxygen Impact Comfort in Contact Lens Wear?: A Review of the Literature. Eye & Contact Lens 33; 2007: 148–155.
11. **D´Souza, S., Tong, L.:** Practical issues concerning tear protein assays in dry eye. Eye Vis (Lond) [online]. November 2014. Dostupné na http://eandv.biomedcentral.com/articles.
12. **Ebihara, N., Funaki, T., Takai, S. et al.:** Tear chymase in vernal keratoconjunctivitidis. Curr Eye Res, 28; 2004: 417–420.
13. **Evans, V., Vockler, C., Friedlander, M. et al.:** Lacryglobin in human tears, a potential marker for cancer. Clin Exp Ophtalmol 29; 2001: 161–163.
14. **Gemenetzi, M., Lotery, A.J.:** Complement pathway biomarkers and age-related macular degeneration. Eye (Lond), 30; 2016: 1–14.
15. **Ghasemi, H.:** Roles of IL-6 in Ocular Inflammation: A Review. Ocul Immunol Inflamm, 25; 2017: 1–14.
16. **Gillan, W.I.T.H.:** Tear biochemistry: a review. S Afr Optom, 69; 2010: 100–106.
17. **Gomes, B.d.A.F., Santhiago, M.R., Magalhães, P. et al.:** Ocular findings in patients with systemic sclerosis. Clinics 66; 2011: 379–385.
18. **Gu, J., Pauer, G.J., Yue, X. et al.:** Proteomic and genomic biomarkers for age -related macular degeneration. Adv Exp Med Biol 664; 2010: 411–417.
19. **Hagan, S., Martin, E., Enríquez-de-Salamanca, A.:** Tear fluid biomarkers in ocular and systemic disease: potential use for predictive, preventive and personalised medicine. EPMA J [online]. Júl, 2016. Dostupné na https://link.springer.com/article/10.1186/s13167-016-0065-3
20. **Hagan, S., Tomlinson, A.:** Tear Fluid Biomarker Profiling: A Review of Multiplex Bead Analysis. Ocul Surf 11; 2013: 219–235.
21. **Holopainen, J.M., Robciuc, A., Cafaro, T.A. et al.:** Pro-Inflammatory Cytokines and Gelatinases in Climatic Droplet Keratopathy. Invest Ophtalmol Vis Sci, 53; 2012: 3527-3535.
22. **Hsu, M.-Y., Chen, S.-J., Chen, K.-H. et al.:** Monitoring VEGF levels with low-volume sampling in major vision-threating disease: age-related macular degeneration and diabetic retinopathy. Lab Chip 15; 2015: 2357–2363.
23. **Ihnatko, R., Edén, U., Lagali, N. et al.:** Analysis of protein composition and protein expression in the tear fluid of patients with congenital aniridia. J Proteimics, 94; 2013: 78–88.
24. **Kalsow, C.M., Reindel, W.T., Merchea, M.M. et al.:** Tear cytokine response to multipurpose solutions for contact lenses. Clin Ophtalmol 7; 2013: 1291–1302.
25. **Kehinde, L.E., Elder, K.S., Fullard, R.J.:** Effect of Daily Versus 30 Day Continuous Contact Lens Wear on Tear Cytokine Levels. Invest Ophtalmol Vis Sci 50; 2009: 5656.
26. **Kim, W.S., Wee, S.W., Lee, S.H. et al.:** Angiogenin for the Diagnosis and Grading of Dry Eye Syndrome. Korean J Ophthalmol, 30; 2016: 163–171.
27. **Lam, H., Bleiden, L., de Paiva, C.S. et al.:** Tear cytokine profiles in dysfunctional tear syndrome. The American Journal of Ophthalmology, 147; 2009: 198–205.
28. **Lambert, N.G., ElShelmani, H., Singh, M.K. et al.:** Risk factors and biomarkers of age-related macular degeneration. Prog Retin Eye Res, 54; 2016: 64–102.
29. **Lebrecht, A., Boehm, D., Schmidt, M. et al.:** Diagnosis of breast cancer by tear proteomic pattern. Cancer Genomics Proteomics, 6; 2009: 177–182.
30. **Leonardi, A.:** Allergy and allergic mediators in tears. Exp Eye Res, 117; 2013: 106–117.
31. **Leonardi, A., Borghesan, F., Faggian, D. et al.:** Microarray-based IgE detection in tears of patients with vernal keratoconjunctivitis. Pediatr Allergy Immunol, 26; 2015: 641–645.
32. **Li, B., Sheng, M., Li, J. et al.:** Tear proteomic analysis of Sjögren syndrome patients with dry eye syndrome by two-dimensional-nano-liquid chromatography coupled with tandem mass spectrometry. Sci Rep [online]. August 2014. Dostupné na http://www.nature.com/articles/srep05772.
33. **Licier, R., Miranda, E., Serrano, H.:** A Quantitative Proteomics Approach to Clinical Research with Non-Traditional Samples. Proteomes [online]. Október, 2016. Dostupné na http://www.mdpi.com/2227-7382/4/4/31.
34. **Malvitte, L., Montange, T., Vejux, A. et al.:** Measurment of inflammatory cytokines by multicytokine assay in tears of patients with glaucoma topically treated with chronic drugs. Br J Ophtalmol, 91; 2007: 29–32
35. Massingale, M.L., Li, X., Vallabhajosyula, M. et al.: Analysis of inflammatory cytokines in the tears of dry eye patients. Cornea, 28; 2009: 1023–1027.
36. **Matsumura, T., Takamura, Y., Tomomatsu, T. et al.:** Changes in Matrix Metalloproteinases in Diabetes Patients´ Tears After Vitrectomy and the Relationship With Corneal Epithelial Disorder. Invest Ophtalmol Vis Sci, 56; 2015: 3559–3564.
37. **Mysona, B.A., Matragoon, S., Stephens, M. et al.:** Imbalance of the Nerve Growth Factor and Its Precursor as a Potential Biomarker for Diabetic Retinopathy. BioMed Res Int [online]. Október, 2014. Dostupné na https://www.hindawi.com/journals/bmri/2015/571456/.
38. **Nishtala, K., Pahuja, N., Rohit, S. et al.:** Tear biomarkers for keratoconus. Eye Vis (Lond) [online]. August, 2016. Dostupné na http://eandv.biomedcentral.com/articles/10.1186/s40662-016-0051-9.
39. **Osaadon, P., Fagan, X.J., Lifshitz, T. et al.:** A review of anti-VEGF agents for proliferative diabetic retinopathy. Eye (Lond), 28; 2014: 510–520.
40. **Park, K.S., Kim, S.S., Kim, J.C. et al.:** Serum and Tear Levels of Nerve Growth Factor in Diabetic Retinopathy Patients. Am J Ophtalmol 145; 2008: 432–437.
41. **Patel, R., Shahane, A.:** The epidemiology of Sjögren's syndrome. Clin Epidemiol [online]. Júl, 2014. Dostupné na https://www.dovepress.com/the-epidemiology-of-sjoumlgrenrsquos-syndrome-peer-reviewed-article-CLEP.
42. **Peral, A., Carracedo, G., Pintor, J.:** Diadenosine polyphosphates in the tears of aniridia patients. Acta Ophtalmol, 93; 2015: 337–342.
43. **Perumal, N., Funke, S., Pfeiffer, N. et al.:** Proteomics analysis of human tears from aqueous-deficient and evaporati-

ve dry eye patients. Scientific Reports [online]. Júl, 2016. Dostupné na http://www.nature.com/articles/srep29629.

44. **Priyadarsini, S., Hjortdal, J., Sarker-Nag, A. et al.:** Gross Cystic Disease Fluid Protein-15/Prolactin-Inducible Protein as a Biomarker for Keratoconus Disease. PLoS One [online]. November, 2014. Dostupné na http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0113310#s2.
45. **Pusparajah, P., Lee, L.-H., Kadir, K.A.:** Molecular Markers of Diabetic Retinopathy: Potential Screening Tool of the Future? Front Physiol [online]. Jún 2016. Dostupné na http://journal.frontiersin.org/article/10.3389/fphys.2016.00200/full.
46. **Rentka, A., Hársfalvi, J., Berta, A. et al.:** Vascular Endothelial Growth Factor in Tear Samples of Patients with Systemic Sclerosis. Mediators Inflamm [online]. Marec, 2015. Dostupné na https://www.hindawi.com/journals/mi/2015/573681/.
47. **Rentka, A., Hársfalvi, J., Szucs, G. et al.:** Membrane array and multiplex bead analysis of tear cytokines in systemic sclerosis. Immunol Res, 64; 2016: 619–626.
48. **Salvisberg, C., Tajouri, N., Hainard, A. et al.:** Exploring the human tear fluid: discovery of new biomarkers in multiple sclerosis. Proteomics Clin Appl 8; 2014: 185-194.
49. **Satici, A., Guzey, M., Dogan, Z. et al.:** Relationship between Tear TNF-alpha, TGF-beta1, and EGF levels and severity of conjunctival cicatrization in patients with inactive trachoma. Ophtalmic Res [online]. December, 2003. Dostupné na https://www.karger.com/Article/Abstract/74067.
50. **Serra, H.M., Beuerman, R.W., Zhou, L. et al.:** Study of N-Linked Glycoproteins in Tears From Patients With Climatic Droplet Keratopathy in Argentina. Invest Ophtalmol Vis Sci, 50; 2009: 5043.
51. **Serra, H.M., Holopainen, J.M., Beuerman, R. et al.:** Climatic droplet keratopathy: an old disease in new clothes. Acta Ophtalmol, 93; 2015: 469–504.
52. **Tailor, R., Gupta, A., Herrick, A. et al.:** Ocular Manifestations of Scleroderma. Survey of Ophtalmology, 54; 2009: 292–304.
53. **Taylor, H.R., Burton, M.J., Haddad, D. et al.:** Trachoma. In The Lancet, 13-19 December 2014. Zborník [online]. 2014 [cit. 22.2.2017]. Dostupné na http://www.sciencedirect.com/science/article/pii/S0140673613621820.
54. **Tong, L., Lan, W., Lim, R.R. et al.:** S100A Proteins as Molecular Targets in the Ocular Surface Inflammatory Diseases. Ocul Surf, 12; 2014: 23-31.
55. **Vesura, P., Bavelloni, A., Grillini, M. et al.:** Diagnostic performance of a tear protein panel in early dry eye. Mol Vis, 19; 2013: 1247–1257.
56. **von Thun und Hohenstein-Blaul, N., Funke, S., Grus, F.H.:** Tears as a source of biomarkers for ocular and systemic disease. Exp Eye Res, 117; 2013:126–137.
57. **Willcox, M.D.P., Zhao, Z., Naduvilath, T. et al.:** Cytokine changes in tears and relationship to contact lens discomfort. Mol Vis, 21; 2015: 293–305.
58. **Wu, K., Zhang, Y.:** Clinical application of tear proteomics: Present and future prospects. Proteomics Clin Appl, 1; 2007: 972–982.
59. https://clinicaltrials.gov/ct2/show/NCT03037463.