NKFIH 112612 “Fibrinolysis at the interface of inflammation and atherosclerosis”
FINAL REPORT

The results of the funded four-year project were published in 17 full-length papers in peer-reviewed journals with a total impact factor of 61.844. In addition, 23 abstracts were presented as talks or posters at national and international scientific congresses organised by the International Society on Thrombosis and Haemostasis, the International Society of Fibrinolysis and Proteolysis and the Hungarian Society on Thrombosis and Haemostasis. The impact of the performed research is reflected in the number of independent citations (149) received for the papers published within the framework of the completed project. Two of the papers (**#1** and **#2**) were ranked among the top 5 % highly cited articles in the first 24 months after publication in the category of Haematology (Scopus database). Based on this achievement, in 2018 the NKFIH awarded a new 2-year research grant (KH_129528) to the principal investigator (PI). **Bernd Engelmann**, the editor of *Thrombosis and Haemostasis*, wrote an editorial comment (***Thrombosis and Haemostasis*** (2015; 113: 1164) on our **paper #2**, in which he highlighted the following seminal contribution of our work: “The study by Varjú et al. (4) adds a new aspect to the procoagulant role of nucleosome components and NETs. These authors show that isolated eukaryotic DNA and histone proteins such as HIIIS enhance the diameter of thrombus-forming fibrin fibers under *in vitro* conditions. Moreover, they are found to decrease the permeability of fibrin clots and to enhance the overall stability of such clots. Mechanistically, these effects are mediated by regulation of endogenous anticoagulant and fibrinolytic pathways. Indeed, histone proteins prevented the inactivation of thrombin by antithrombin and DNA reduced plasminogen activation. Furthermore, NETs released from isolated neutrophils suppressed t-PA-promoted fibrinolysis. Together with earlier work, these results further corroborate the conclusion that extracellular nucleosomes and their major components target the coagulation system at different levels and that their overall effect is to enhance fibrin formation.”

Paper#2 extends the earlier observations that isolated DNA and histones promote thrombus formation and render fibrin clots more resistant to mechanical forces and tissue-type plasminogen activator (tPA)-induced enzymatic digestion to a physiologically more relevant setting. In an environment including plasma clots and neutrophil extracellular traps (NETs)-forming neutrophils a range of techniques was employed including imaging (scanning electron microscopy (SEM), confocal laser microscopy, and photoscanning of macroscopic lysis fronts), clot permeability measurements, turbidimetric lysis and enzyme inactivation assays. Addition of DNA and histones increased the median fibrin fiber diameter in plasma clots from 108 to 121 and 119 nm, respectively, and decreased their permeability constant from 6.4 to 3.1 and 3.7×10^{-9} cm^2. Histones effectively protected thrombin from antithrombin-induced inactivation, while DNA inhibited plasminogen activation on the surface of plasma clots and their plasmin-induced resolution by 20 and 40%, respectively. DNA and histones, as well as NETs secreted by phorbol-myristate-acetate-activated neutrophils, slowed down the tPA-driven lysis of plasma clots and the latter effect could be reversed by the addition of DNase. SEM images taken after complete digestion of fibrin in NET-containing plasma clots evidenced retained NET scaffold that was absent in DNase-treated clots. Our results show that DNA and histones alter the fibrin architecture in plasma clots, while NETs contribute to a decreased lytic susceptibility that can be overcome by DNase.

Because of this action of NETs to promote coagulation and increase the mechanical and lytic stability of fibrin, we explored additional approaches to destabilise NETs as a tool to reduce thrombosis and treat sepsis (**paper#3**). Because heparinoids bind histones, we performed quantitative studies in plasma and purified systems to better understand the physiological consequences of these interactions in terms of fibrin stability. Unfractionated heparin (UFH) was investigated by activated partial thromboplastin time (APTT) and alongside low molecular weight heparins (LMWH) in purified systems with thrombin or factor Xa (FXa) and antithrombin (AT) to measure the sensitivity of UFH or LMWH to histones. A method was developed to assess the effectiveness of DNA and non-anticoagulant heparinoids as anti-histones. Histones effectively neutralised UFH, the IC50 value for neutralisation of 0.2 IU/ml UFH was 1.8 μg/ml histones in APTT and 4.6 μg/ml against 0.6 IU/ml UFH in a purified system. Histones also inhibited the activities of

LMWHs with thrombin (IC50 6.1 and 11.0 µg/ml histones, for different LMWHs) or FXa (IC50 7.8 and 7.0 µg/ml histones). Direct interactions of UFH and LMWH with DNA and histones were explored by surface plasmon resonance, while rheology studies showed complex effects of histones, UFH and LMWH on clot resilience that was based on modified fibrin structure demonstrated by SEM, clot permeation and small angle X-ray scattering (SAXS). These studies showed that anticoagulation by UFH and LMWH could be compromised by high affinity binding to circulating histones even in the presence of DNA. Our study provided a definite proof that a complete understanding of the effects of histones, DNA and heparins on the haemostatic system must include an appreciation of direct effects on fibrin and clot structure.

We demonstrated additional inflammation-related factors in the extracellular matrix that modify the structural, mechanical and lytic features of fibrin, expanding the scope of our work to the interference of hyaluronic acid (HA), a large, non-sulfated glycosaminoglycan that is ubiquitously present in extracellular matrices with the structure and degradation of fibrin. This aspect of our work shed light on the consequences of co-localization of HA and fibrin at sites of primary physiological and pathological relevance (during wound healing, in arterial restenotic lesions and eroded atherosclerotic plaques, microenvironments of many solid tumors). In **paper#4** we aimed to characterize the structure of composite fibrin-HA clots with SEM, pressure-driven permeation and SAXS, their viscoelastic properties with oscillation rheometer and the efficiency of fibrinolysis in these clots with kinetic turbidimetric and chromogenic assays for dissolution of fibrin and plasminogen activation by tPA. Fibrin formed in the presence of native (1,500 kDa) HA and its 500 kDa fragments had thicker fibers and larger pores according to the SEM and clot permeation data, whereas the 25 kDa HA fragments had only minor effects. SAXS evidenced a mild disarrangement of protofibrils. These structural alterations suggest that HA modifies the pattern of fibrin polymerization favoring lateral association of protofibrils over formation of branching points. Rheometer data evidenced softer fibrin structures formed with 1,500 kDa and 500 kDa HA and these clots presented with lower dynamic viscosity values and lower critical stress values at gel/fluid transition. tPA-catalyzed plasminogen activation was markedly inhibited by HA, both in free solution and on the surface of fibrin clots, in the presence and in the absence of 6-aminohexanoate suggesting a kringle-independent mechanism. HA of 1,500 and 500 kDa size prolonged clot lysis with both plasmin and tPA and this inhibition was kringle-mediated, because 6-aminohexanoate abolished it and it was not observed with des-(kringle1-4)-plasmin. Our data suggest that high-molecular weight HA may favour fibrin network preservation, but could lose its effectiveness upon fragmentation at sites of tissue remodelling and inflammation.

Because free fatty acids are released from platelets and inflammatory cells at the stage of thrombus formation, but their effects on fibrin formation are largely unexplored, we addressed the kinetic effects of fatty acids on thrombin activity, as well as the structural and mechanical properties of the resultant fibrin clots (**paper#5**). In turbidimetric assays of fibrin generation, oleate and stearate at physiologically relevant concentrations (60-600 µM) produced a bell-shaped inhibitory dose response, increasing 10- to 30-fold the time to half-maximal clotting. Oleate inhibited the thrombin activity on a short peptide substrate according to a mixed-type inhibitor pattern (a 9-fold increase of the Michaelis constant and a 20 % decrease of the catalytic constant). Morphometric analysis of SEM images of fibrin showed a 73 % increase in the median fiber diameter in the presence of stearate and a 20 % decrease in the presence of oleate. Evaluation of the viscoelastic parameters of the clots with oscillation rheometer indicated decreased rigidity, higher deformability and decreased internal resistance to shear stress of fibrin containing oleate or stearate. Our study provided convincing evidence that free fatty acids (at concentrations comparable to those reported in thrombi) reduce the mechanical stability of fibrin through modulation of thrombin activity and the pattern of fibrin assembly.

Our work on the role of cyclophilin D (CypD) reported in **paper#6** revealed that CypD moderates platelet adhesion, but enhances the lytic resistance of fibrin formed around platelets. In the course of thrombosis, platelets are exposed to a variety of activating stimuli classified as ‘strong’ (e.g. thrombin and collagen) or ‘mild’ (e.g. ADP). In response, activated platelets adhere to injured vasculature, aggregate, and stabilise the three-dimensional fibrin scaffold of the expanding thrombus. Since ‘strong’ stimuli also induce opening of the mitochondrial permeability transition pore (MPTP) in

platelets, the MPTP-enhancer CypD has been suggested as a critical pharmacological target to influence thrombosis. However, it was unknown how CypD influences platelet-driven clot stabilisation against enzymatic breakdown (fibrinolysis). Our work showed that treatment of human platelets with Cyclosporine A (a cyclophilin-inhibitor) boosted ADP-induced adhesion and aggregation, while genetic ablation of CypD in murine platelets enhanced adhesion but not aggregation. We also found that platelets lacking CypD preserved their integrity in a fibrin environment, and lost their ability to render clots resistant against fibrinolysis. Our results indicate that CypD has opposing haemostatic roles depending on the stimulus and stage of platelet activation. In addition our study identified several mechanistic factors that can reverse the anti-fibrinolytic effects of CypD. (i) Electron microscopy showed preserved ultrastructural integrity of $CypD^{-/-}$ platelets, implying limited release of anti-fibrinolytic constituents (e.g. phospholipids, myosin). (ii) The presence of $CypD^{-/-}$ platelets resulted in larger fibrin fibre diameters compared to WT platelets with a stronger effect under oxidative stress. Since fibre diameter is an important determinant of tPA-induced lysis (coarse meshwork with thicker fibres being more susceptible), this finding might also contribute to the observed differences in the lysis of clots containing WT and $CypD^{-/-}$ platelets.

In addition to the investigation of the basic mechanisms contributing to the stability of thrombi, another focus of the research in this project was to investigate malfunctions in the fibrinolysis pathway related to selected disease states with inflammatory component. A comprehensive overview of the bleeding disorders stemming from abnormal fibrinolysis was published in our review **paper#7**. Our original research data provided evidence for the role of disturbed fibrinolysis in the pathomechanism of dermatitis herpetiformis (DH) (**papers#8&9**). Because high prevalence of cryofibrinogenaemia has been observed in plasma of untreated DH patients and the pathological immunoglobulin A (IgA) and transglutaminase 3 (TG3) deposits are found to co-localize with fibrin and fibrinogen in the skin lesions, we studied the fibrinolytic potential in plasma of untreated, dapsone and or/ gluten-free diet treated DH patients as well as the in vitro effect of dapsone on the fibrinolytic profile. A significantly prolonged clot lysis time was detected in untreated DH patients. The turbidity values of DH plasma clots indicated an altered fibrin structure that was also confirmed by SEM: significantly thicker fibrin fibers were observed in untreated, TG3 antibody positive DH patients compared to healthy controls, whereas the fiber diameters of dapsone-treated patients were similar or thinner than the control values. In line with the structural changes of fibrin, the fibrinolytic profile of patients under dapsone treatment approached the control values. This study revealed that the fibrinolytic potential was impaired in the plasma of untreated DH patients, whereas dapsone corrected the fibrinolytic defect and suggested a pathogenic role for plasma-derived factors in the development of skin symptoms in active DH. In addition, we could show that circulating anti-TG3 IgA-type antibodies are deposited in the DH skin lesions raising the possibility for local disturbances of fibrinolysis similar to those observed by us in the systemic blood samples of the DH patients.

Because of the dynamic interactions of fibrin and extracellular matrix components in inflammation and wound healing, exploring the pathomechanism of diseases related to the catabolism of fibrin we expanded the scope of our work to the stability of additional components of the extracellular matrix. Thus, in **papers#10** we addressed the turnover of collagen in keratoconus disease (KC), which is characterized by thinning and deformation of the cornea, but its aetiology remains unknown. Seventy percent of the corneal stroma consists of collagen, which is composed of three intertwined polypeptide chains with glycine-hydroxyproline-proline repeats along their sequence. Arginase is a cytoplasmatic enzyme and catalyzes the conversion of arginine to urea and ornithine, which serves as a precursor for the endogenous synthesis of proline and (post-translationally) hydroxyproline in tissues with limited blood supply (such as the cornea). We addressed the collagen turnover by measuring arginase activity in cultured normal and KC-keratocytes and hydroxyproline concentration in the conditioned culture medium. We demonstrated suppressed arginase activity in the metabolic program of cultured KC keratocytes, which results in impaired collagen synthesis, evidenced by reduced hydroxyproline synthesis in the keratocyte culture. Thus, we identified the metabolic reprogramming of KC- keratocytes as a pathogenic factor in KC disease.

A translational aspect was added to our research by the work on the development of protease-loaded liposomes reported in **papers#11**. Protease encapsulation and its targeted release in thrombi may contribute to the reduction of haemorrhagic complications of thrombolysis. We aimed to prepare sterically stabilized trypsin-loaded liposomes (SSLT) and characterize their structure and fibrinolytic efficiency. Hydrogenated soybean phosphatidylcholine-based SSLT were prepared and their structure was studied by transmission electron microscopy combined with freeze fracture (FF-TEM), Fourier transform infrared spectroscopy (FT-IR) and SAXS. Fibrinolytic activity was examined at 45, 37 or 24°C on fibrin or plasma clots with turbidimetric and permeation-driven lysis assays. Trypsin was shown to be attached to the inner surface of vesicles (SAXS, FF-TEM) close to the lipid hydrophilic/hydrophobic interface (FT-IR). The thermosensitivity of SSLT was evidenced by enhanced fibrinolysis at 45°C: time to reduce the maximal turbidity to 20% decreased by 8.6% compared to 37°C and fibrin degradation product concentration in the permeation lysis assay was 7-fold higher than at 24˚C. SSLT exerted its fibrinolytic action on fibrin clots under both static and dynamic conditions, whereas plasma clot dissolution was observed only in the permeation-driven assay. The improved fibrinolytic efficiency of SSLT under dynamic conditions suggests that they may serve as a novel therapeutic candidate for dissolution of intravascular thrombi, which are typically exposed to permeation forces.

Although most of the work performed within the framework of the project is basic science research, several results bear immediate clinical relevance. In our **paper #12** we performed analysis of the relationships between clinical data and structural characteristics of thrombi. Thrombus samples removed by percutaneous coronary intervention (thrombus aspiration) following acute myocardial infarction (n=101) or surgical open repair of large peripheral arteries (n=50) were processed for scanning electron microscopy or for indirect immunostaining for fibrin and platelet receptor GpIIb/IIIa. We focused on the influence of smoking and accompanying pathological states on the composition of coronary and peripheral arterial thrombi. A significant difference was found in fibrin content of coronary thrombi between smokers and non-smokers. In line with this data, fluorescent fibrin/platelet coverage ratio was significantly lower in non-smokers. No direct influence of smoking was observed on clot RBC occupancy. A non-linear positive dependence of thrombus platelet content on systemic platelet count was observed in both coronary and peripheral thrombi. While the correlation was altered by gender and anti-platelet pre-treatment in peripheral thrombosis patients, the association found in coronary thrombi was stronger in active and former smokers and in patients with dyslipidaemia. Higher intrathrombotic platelet content tends to be accompanied by thinner fibrin fibers in peripheral thrombi, which correlation is remarkably stronger when dyslipidaemia is present. By multivariable regression analysis, a strong correlation of haematocrit, patient age and clot RBC content was found among hypertensive patients. Leukocyte and RBC count jointly influenced clot platelet content, especially in female, atherosclerotic and non-diabetic peripheral patients. Our new analysis revealed that cigarette smoking and accompanying pathological conditions have pronounced effects on thrombus composition. These observations may help to understand how these conditions relate to arterial thrombosis.

In the second publication of direct clinical relevance (**paper#13**) we reported results on the presence of NETs in the structure of thrombi retrieved during interventional treatment of ischemic vascular disease states. The ultrastructure and cellular composition of thrombi has a profound effect on the outcome of coronary (CAD), peripheral artery disease (PAD) and acute ischemic stroke (AIS). We investigated the NET-related structural features of thrombi retrieved from different arterial localizations and their interrelations with routinely available clinical data. We reported the data gained from thrombi extracted from CAD (n=66), PAD (n=64) or AIS (n=78) patients that were processed for scanning electron microscopy, (immune)stained for fibrin, citrullinated histone H3 (cH3) and extracellular DNA. Fibrin fiber diameter, cellular components, DNA and cH3 were measured and analysed in relation to clinical parameters. DNA was most abundant in PAD thrombi showing a 2.5-fold higher DNA/fibrin ratio than AIS, whereas cH3 antigen was unvaryingly present at all locations. The NET content of thrombi correlated parabolically with systemic inflammatory markers and positively with patients' age. The median platelet content was lower in PAD (2.2%) than in either CAD

(3.1%) or AIS (3.9%) and thrombi from smokers contained less platelets than non-smokers. Fibrin fibers were significantly thicker in male patients with CAD (median fiber diameter 76.3 nm) compared to PAD (62.1 nm) or AIS (64.1 nm) and their diameter correlated parabolically with systemic inflammatory markers. An interesting observation in this study was that oral anticoagulant medications were associated with an increase in relative cH3 content in thrombi which could be attributed to a decrease in thrombin generation (secondary to anticoagulant use), a protease that can digest histones. The observed NET-related variations in thrombus structure shed light on novel determinants of thrombus stability that eventually affect both the spontaneous progress and therapeutic outcome of ischemic arterial diseases.

A new approach was developed for the statistical treatment of the data from the clinical studies. These were analysed with statistical hypothesis tests for differences between groups of patients with common clinical features and regression analysis for correlation between measured data and clinical parameters. The statistical analysis was used to draw general conclusions on the characteristics of the disease. However, a single thrombus from a single patient is exposed to effects that vary in space (e.g. blood flow in different regions of the thrombus). This results in a high degree of biological diversity reflected in the heterogeneity of the data measured in different areas of a thrombus. A statistical analytical procedure of hypothesis testing or regression analysis could account for this heterogeneity treating all data as separate observations characterizing the disease, if the intraindividual heterogeneity is neglected. However, because of the different size of the thrombus samples and the consequent difference in the number of available measured data from each patient, this approach distorts the role of interindiviual differences ascribing higher weight for patients with larger sets of measured data. If the interindividual differences are to be considered as a factor in the disease mechanism, all intraindividual data should be taken into account in the statistical analysis as a single observation originating as a dataset composed of discrete subsets of actually measured data with appropriate weighing factor reflecting the total number of measurements taken from each thrombus. For example, the red blood cell occupancy in two different sections of the same thrombus can vary between 7 and 21 % (**paper#13**). If these two data are considered as separate observations, this increases the number of clots with thin or thick fibers taken into consideration. However, the mechanism of the disease in the specific patient is better reflected by a single observation combining the weighed occupancy in a single thrombus. This type of analysis forms the basis of the fuzzy statistical approach that was used in our work and its theoretical background was elaborated in **paper#14**.

The techniques developed for the evaluation of fibrin ultrastructure proved to be a valuable tool for the examination of the lens capsule structure in the course of ophthalmologic surgery (**papers #15-17**). Although apparently not related to the topic of the project, these results were possible only due to the experience acquired in the sample processing of fibrin clots and thrombi for SEM within the framework of this project.

The support from NKIFH definitely contributed to the sustainability of the human resources at the hosting research group, the development of their professional career and international networking. The funded research allowed two PhD students (Farkas Veronika és Farkas Ádám) to meet the research requirements for a PhD degree. The NKFIH funding contributed to the continuing collaboration with the research teams of Dr Colin Longstaff (National Institute for Biological Standards and Control, South Mimms, UK) and Prof Kiril Tenekedjiev (University of Tasmania, Launceston, Australia) exemplified by co-authored publications (**papers #1,2,3,5,7,12,13,14**). The international visibility of the research supported by NKFIH was promoted by the invited talks, which were delivered by the PI of the project at the XXVth Congress of the International Society on Thrombosis and Haemostasis, Toronto June 20-June 25, 2015 and at the 14th Congress of the World Federation of Interventional and Therapeutic Neuroradiology, Budapest, 16-19. Oct., 2017. During the project the PI was repeatedly elected to act as a Co-Chair of the Fibrinolysis Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, as a Council Member of the International Society of Fibrinolysis and Proteolysis and as an Editorial Board Member of the *Journal of Thrombosis and Haemostasis* and of the *Thrombosis Research* journals.

Full-length peer-reviewed papers published with the funding support from NKFIH

1. Longstaff C; **Kolev K**. Basic mechanisms and regulation of fibrinolysis. *J Thromb Haemost* 2015; 13: S98-S105

IF5.550

2. Varjú I; Longstaff C; Szabó L; Farkas AZ; Varga-Szabó VJ; Tanka-Salamon A; Machovich R; **Kolev K**. DNA, histones and neutrophil extracellular traps exert anti-fibrinolytic effects in a plasma environment. *Thromb Haemost* 2015;113(6): 1289-1298

IF5.760

3. Longstaff C; Hogwood J; Gray E; Komorowicz E; Varjú I; Varga Z; **Kolev K**. DNA, Neutralization of the anti-coagulant effects of heparin by histones in blood plasma and purified systems. *Thromb Haemost* 2016; 115(3): 591-599

IF5.627

4. Komorowicz E; Balázs N; Varga Z; Szabó L; Bóta A; **Kolev K**. Hyaluronic acid decreases the mechanical stability, but increases the lytic resistance of fibrin matrices. *Matrix Biol* 2017; 63: 55-68

IF8.136

5. Tanka-Salamon A; Komorowicz E; Szabó L; Tenekedjiev K; **Kolev K**. Free Fatty Acids Modulate Thrombin Mediated Fibrin Generation Resulting In Less Stable Clots. *PlosOne* 2016; 11: e0167806

IF2.806

6. Varjú I; Farkas VJ; Kőhidai L; Szabó L; Farkas ÁZ; Polgár L; Chinopoulos C; **Kolev K**. Functional cyclophilin D moderates platelet adhesion, but enhances the lytic resistance of fibrin. *Sci Rep* 2018;8(1):5366

IF4.122

7. **Kolev K**; Longstaff C. Bleeding related to disturbed fibrinolysis. *Brit J Haematol* 2016; 175: 12-23

IF5.670

8. Görög A; Németh K; **Kolev K**; Zone JJ; Mayer B; Silló P; Bognár P; Kárpáti S. Circulating Transglutaminase 3 – Immunoglobulin A immune complexes in dermatitis herpetiformis. *J Invest Dermatol* 2016; 136(8):1729-31

IF6.287

9. Görög A; Németh K; Szabó L; Mayer B; Silló P; **Kolev K**; Kárpáti S. Decreased fibrinolytic potential and morphological changes of fibrin structure in dermatitis herpetiformis. *J Dermatol Sci* 2016; 84(1):17-23

IF3.733

10. Stachon T; **Kolev K**; Flaskó Z; Seitz B; Langenbucher A; Szentmáry N. Arginase activity, urea concentration and hydroxyproline synthesis are reduced in *keratoconus* keratocytes. *Graefe's Arch Clin Exp Ophthalmol* 2017; 255(1):91-97

IF2.249

11. Tanka-Salamon A; Bóta A; Wacha A; Mihály J; Lovas M; **Kolev K**. Structure and function of trypsin-loaded fibrinolytic liposomes. *BioMed Res Int* 2017 (2017), Article ID 5130495

IF2.583

12. Kovács A; Sótonyi P; Nagy AI; Tenekedjiev K; Wohner N; Komorowicz E; Kovács E; Nikolova N; Szabó L; Kovalszky I; Machovich R; Szelid Z; Becker D; Merkely B; **Kolev K**. Ultrastructure and

composition of thrombi in coronary and peripheral artery disease: correlations with clinical and laboratory findings. *Thromb Res* 2015; 135: 760-766

IF2.427

13. Farkas ÁZ; Farkas VJ; Gubucz I; Szabó L; Bálint K; Tenekedjiev K; Nagy AI; Sótonyi P; Hidi L; Nagy Z; Szikora I; Merkely B; **Kolev K**. Neutrophil extracellular traps in thrombi retrieved during interventional treatment of ischemic arterial diseases. *Thromb Res* 2019; 174: *in press* doi 10.1016/j.thromres.2019.01.006

IF2.779

14. Nikolova ND; Chai S; Ivanova SD; **Kolev K**; Tenekedjiev K. Bootstrap Kuiper Testing of the Identity of 1D Continuous Distributions using Fuzzy Samples. *Int J Comput Int Sys* 2015; 8(Suppl.2): 63-75 doi: 10.1080/18756891.2015.1129592

IF0.391

15. Sándor GL; Kiss Z; Bocskai Z; **Kolev K**; Takács AI; Juhász E; Kránitz K; Tóth G; Gyenes A; Bojtár I; Juhász T; Nagy ZZ. Comparison of the mechanical properties of anterior lens capsule following femtosecond laser capsulotomy at different energy settings. *J Refract Surg* 2015; **31**(3):153-157

IF2.781

16. Ecsedy M; Sandor GL; Takacs AI; Kranitz K; Kiss Z; **Kolev K**; Nagy ZZ. Femtosecond laser-assisted cataract surgery in Alport syndrome with anterior lenticonus. *Eur J Ophtalm* 2015; doi: 10.5301/ejo.5000603

IF1.058

17. Sándor GL; Kiss Z; Bocskai ZI; **Kolev K**; Takács AI; Juhász É; Kránitz K; Tóth G; Gyenes A; Bojtár I; Juhász T; Nagy ZZ. A szemlencse elülső tokjának biomechanikai vizsgálata manuális capsulorhexis és femtoszekundumos lézeres capsulotomia után. *Szemészet* 2015; 152: (3) 122-130