

Activities during the the project were essentially proceeding as originally planned in the Workplan. Within the frame of the project 12 in extenso publications were published in well-rated international journals (NKFI support acknowledged in 11 papers, total IF: 31.408). Altogether 18 conference proceedings/abstracts were published and the results of the study were successfully presented at over 20 international/national congresses. During the project 4 PhD theses were published based on findings of the project (NKFI acknowledged in all 4 thesis books).

Detailed report:

I. Inclusion of patients and controls:

By the end of the project a total of 137 patients were enrolled (116 AF patients and 21 non-AF controls), which exceeded expectations of the Workplan (by the 4th year a total of 110 patients were planned to be included). Besides the 21 control individuals undergoing ablation procedure included in the study, an appropriately tested population control group including 140 individuals was applied for comparisons. Baseline demographic characteristics of the patient cohort: age: mean: 54.6±12.4 years; male: 80/137 (58.4%), BMI: 28.9 (IQR:25.9-32.1), arterial hypertension: 75/137 (54.8%), hyperlipidemia: 13/137 (9.5%), diabetes mellitus: 11/137 (8.0%); active smoker: 12/122 (8.8%). A number of clinical data were registered (CHADS, CHAD VASC score, EHRA score, TTE and TEE parameters, left atrium diameter and length, dilatation of the left ventricle, ejection fraction%, LAA flow, spontaneous echo contrast). Carotid ultrasound examination was performed before or shortly after the ablation procedure to evaluate significant asymptomatic carotid stenosis. The measurement of cerebral microembolization during the ablation procedure using transcranial Doppler was also carried out in a subset of the patient population. Patients were followed for the re-appearance of the arrhythmia or development of complications such as stroke. Patients suffering stroke were examined and treated according to standard protocols. Imaging data of post-stroke patients was included in two manuscripts describing a functional magnetic resonance imaging (fMRI)-based stroke rehabilitation approach (Aranyi et al, J Neuroimaging 2016;00:1-12. IF: 1.664. NKFI 109712 acknowledged; Vér et al Eur Neurol 2016; 76 (3-4):132-142. IF: 1.697, NKFI 109712 acknowledged.)

II. Transcatheter ablation techniques:

Procedures were performed at the Department of Cardiology, University of Debrecen. Pulmonary vein isolation (PVI) was performed in all patients (n=137) using either cryoballoon (CB), phased radiofrequency technology with the circular, multipolar pulmonary vein ablation catheter (PVAC) or point by point radiofrequency ablation technology. In 2016 a new guideline was introduced for the management of AF, which gave us the opportunity from the beginning of 2017 to investigate patients that are undergoing ablation procedures while on anticoagulation therapy (a modification of ethics approval was attained in 2017). This created a new line of research in which the efficacy of various anticoagulation strategies could be compared during the PVI .

III. Blood sampling:

Blood sampling of all patients was performed from venous and arterial blood samples drawn from the femoral vein (FV) and the left atrium (LA) before and after the transcatheter ablation procedure. Moreover, of the first 24 patients and 13 control individuals, blood sampling was also performed from the left atrial appendage (LAA).

Results of hemostasis and other parameters were evaluated according to the site of sampling, the difference between patient and control cohort and the type of ablation procedure (CB or PVAC). From the beginning of 2017 another arm was introduced in the study where patients on various oral anticoagulants (direct oral anticoagulants:DOACs or vitamin K antagonist therapy:VKA) were also included in the project, the effect of these substances were compared on various hemostasis/fibrinolysis and endothel cell damage markers.

III. Determination of specific hemostasis and fibrinolytic factors and statistical analysis

All laboratory measurements were carried out at the University of Debrecen, Division of Clinical Laboratory Sciences (previously Clinical Research Center). As planned in the Workplan, over 20 hemostasis parameters were measured from each blood sample (137 patients x 2 samples x 20 tests>5420 measurements). In addition to the planned investigations, soluble E-selectin (sE-sel) and VCAM-1 as markers of endothelial damage and the highly researched, relatively new test of thrombin generation were also assessed. CRP, complete lipid profile, NT-proBNP were measured from peripheral venous blood samples. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS 22.0, Chicago, IL) with the following major statistical methods: Spearman's bivariate correlation, multivariate logistic regression analysis, Student's t test/Mann-Whitney U test, ANOVA/Kruskal-Wallis analysis, contingency tables, χ^2 test.

IV. Results and publications according to various aims of the study:

1/ The presence of elevated levels of coagulation factors and fibrinolytic markers in patients with atrial fibrillation

Aims: to identify intracardiac hemostasis abnormalities, which are associated with AF and increase the risk of stroke.

Results: Levels of FVIII and VWF were significantly elevated in the samples obtained from the femoral vein (FV) and LA of AF patients as compared to controls. FVIII and VWF levels showed good correlation, suggesting that they are in complexed form. TAT complex and FM levels were significantly elevated in the LA and the LAA as compared to FV samples in case of both groups. No significant difference was found between sample types and patient groups for FXIII, FVIIa-AT, PAI-1 activity/antigen, α 2PI, plasminogen, fibrinogen levels.

Conclusions: AF patients have elevated FVIII and VWF levels, most likely due to endothelial damage. Local hemostasis activation in the LA and LAA could be demonstrated by elevated TAT complex and FM levels.

Results were presented at national and international congresses. In 2017 a manuscript was prepared and published:

Tóth NK et al. Intracardiac hemostasis and fibrinolysis parameters in patients with atrial fibrillation. *Biomed Res Int* 2017; 2017:3678017. IF: 2.583. NKFI 109712 acknowledged.

Elevated FVIII and VWF levels were associated with stroke severity and outcomes. Results on this arm of the study were published in *Front Neurol* in 2018.

Tóth NK et al. Elevated factor VIII and VWF levels predict unfavorable outcome in stroke patients treated with intravenous thrombolysis. *Front Neurol* 2018; 8:e721. IF(2017): 3.508. NKFI 109712 acknowledged.

2/ Biological markers of atrial fibrillation; association with the occurrence, severity and outcome of stroke

International groups and our group have also observed that paroxysmal AF could be responsible for a significant number of cryptogenic stroke events. In this year of the project, our group has published a comprehensive review on the most recent advances in the research on arrhythmia markers. Various potential biological markers were described in detail in the published paper: Szegedi I, Szapáry L, Csécei P, Csanádi Z, Csiba L. Potential biological markers of atrial fibrillation: a chance to prevent cryptogenic stroke. *Biomed Res In* 2017; 2017: 8153024. IF: 2.583. NKFI 109712 acknowledged.

A potential marker to prove hemostasis activation in AF is the relatively new, global hemostasis test, the thrombin generation test (TGT). We aimed to find out whether TGT parameters are related to the presence of fibrillation, clinical scores, occurrence of stroke, severity of stroke and outcome of thrombolytic therapy in stroke patients. All enrolled patients were included and TGT parameters were compared from blood samples taken from the FV and the LA. Although TGT parameters were not significantly different at these sites, a difference was shown as compared to non-AF controls and results correlated with the number of cerebral microemboli detected during the ablation procedure. A manuscript is currently being prepared on these results. TGT is altered in case of cardioembolic stroke: when we studied TGT in a cohort of stroke patients, endogenous thrombin potential (ETP) and peak thrombin were significantly lower in those with cardioembolic ischemic stroke. A multiple logistic regression model revealed that an ETP result in the lower quartile is an independent predictor of mortality within the first two weeks (OR:6.03; 95%CI: 1.2-30.16, $p<0.05$) and 3 months after the event (OR:5.28; 95%CI:1.27-21.86, $p<0.05$). Results were published in the journal of Plos One (Hudák et al, Low thrombin generation predicts poor prognosis in ischemic stroke patients after thrombolysis, *Plos One*, 2017; e010477. IF: 2.766. NKFI 109712 acknowledged.) As thrombin generation is highly affected by antiplatelet agents, the efficacy of antiplatelet therapy was tested in patients and results were published (Bagoly Z, Homoródi N, Kovács EG, Sarkady F, Csiba L, Édes I, Muszbek L. How to test the effect of aspirin and clopidogrel in patients on dual antiplatelet therapy? *Platelets* 2016; 27: 59-65. IF: 2.465. NKFI 109712 acknowledged.)

3/ Local changes in hemostatic and fibrinolytic parameters in patients with atrial fibrillation during transcatheter ablation procedures

Aims: To find out whether ABL is associated with local, intracardiac alterations of hemostasis/fibrinolysis or with endothelial damage and to analyze different ABL techniques and different anticoagulation strategies in this respect.

Results: When LA samples of pre- and post-ABL were compared, significantly lower FXIII, plasminogen, PAI-1 activities and FM levels as well as markedly elevated D-dimer levels were found post-ABL, suggesting increased clotting and fibrinolysis. When the various ABL technologies were compared, no significant differences were found in FXIII, PAP complex levels, sVCAM-1 or sEsel levels.

Conclusions: Our results confirm that any pulmonary vein techniques used activate the coagulation cascade and causes significant endothelium activation despite of effective vitamin-K antagonist therapy and high dose of heparin administration during the procedure. There was no significant difference between cryoballoon, phased radiofrekvency and irrigated radiofrequency techniques. Results were presented at various international/national meetings, the poster summarizing these results received

the prestigious Paul Dudley White Award of the American Heart Association Annual Meeting held at Chicago in 2018. A manuscript has been prepared from these results (Hajas O et al. Intracardiac changes of fibrinolysis and endothel activation parameters related to atrial fibrillation ablation with different techniques.) and has been submitted to the journal of Heart Rhythm. Micro-embolus generation as detected by transcranial Doppler during PVAC significantly correlated with new silent cerebral ischemia, revealed by diffusion-weighted magnetic resonance imaging. Results were published in *Cardiol J* (Nagy-Baló E et al. Cerebral micro-embolization during pulmonary vein isolation: relation to post-ablation silent cerebral ischemia. *Cardiol J*. 2017; 24: 234-241. IF: 1.339. NKFI 109712 acknowledged.) The influence of other common vascular risk factors in AF (e.g. hypertension, etc). which potentially influence cognition were discussed in a paper published by our group in *CNS Neurol Disord Drug Targets* (Czuriga-Kovács KR et al, 2016; 15(6):690-8, IF: 2.506. NKFI 109712 acknowledged.)

In the last year of the study, 46 patients on various oral anticoagulants undergoing cryoballoon ablation were also included in the study and their results were evaluated most recently. Patients were grouped according to the oral anticoagulant strategy used: 1/ interrupted VKA, 2/ un-interrupted VKA 3/ NOAC therapy. Blood samples taken before and after ablation were analyzed for a large set of hemostasis factors. D-dimer levels were significantly lower in case of NOAC therapy before and after ablation as compared to other strategies (median values before and after ablation: interrupted VKA: 0,48 and 1,09 mgFEU/L; un-interrupted VKA: 0,33 and 0,74 mgFEU/L, NOAC: 0,10 and 0,27 mgFEU/L, $p < 0.001$ interrupted VKA vs. NOAC; $p < 0.01$ un-interrupted VKA vs. NOAC). PAP complex results showed similar tendency. A manuscript on the comparison of the effect of various anticoagulants during ablation procedures is currently being prepared and we plan to submit the results to a highly recognized journal in the field of cardiology.