Final report of PD111929:

The project proceeded essentially as planned in the Workplan, moreover, additional work has been done. Altogether 10 manuscripts were published within the frame of the PD111929 grant in well-recognized international Q1/Q2 journals (total IF: 31.958). In all papers the grant was acknowledged; in 8 of these papers the PI is first or last/corresponding author (2 first;6 corresponding).

The project supported the PhD thesis of 2 students (the grant is acknowledged in both works, the applicant is the supervisor of 1 of the students). The project also supported the MSc and MD thesis of 2 students (the grant is acknowledged in both works, the PI is the supervisor of both students).

Detailed report:

I. Patient enrollment in the clinical study

By the end of the study, blood samples were taken from 253 acute ischemic stroke (AIS) patients, of which 132 patients received i.v. thrombolytic therapy and were included in the study, which is in line with the Workplan. Baseline clinical and demographic characteristics: age: mean:69.0±12.2 years; male gender:77/132 (58.3%), arterial hypertension:101/132 (76.5%), presence of atrial fibrillation:35/132 (26.5%), hyperlipidemia:82/132 (62.1%), diabetes mellitus:40/132 (30.3%), mean time from symptom onset to treatment with rt-PA: 160±46 min. Neurological deficit of patients was determined using the National Institute of Health Stroke Scale (NIHSS) on admission, 2h, 24h and 7 days post-lysis. Stroke etiology was classified using the TOAST criteria. CT images taken before and 24h after thrombolysis were analyzed and the ASPECT scores were calculated. Patients were followed and their neurological status was assessed by the modified Rankin Scale (mRS) at 90 days post-event. Median NIHSS on admission was 8 (IQR:5-14). Median ASPECT scores before and 24h after thrombolysis were 10 (range:10-7) and 9 (range:10-0), respectively. Etiology of stroke was most commonly large-vessel disease (n=49, 37.1%), followed by 27 (20.5%) patients with cardioembolic stroke.

The following outcomes were investigated:

1/short-term functional outcome (by day 7 post-event; unfavorable short-term outcome was defined as an increase in NIHSS score by at least 4 points),

2/long-term functional outcome (by day 90 post-event; unfavorable long-term functional outcome was defined as an mRS score greater than 1),

3/stroke-associated mortality by day 7, day 14 and day 90 post-event

4/the presence of therapy-related intracranial bleeding (according to ECASS II)

Outcome results:

	Values n (%)
Number of patients	132
Short-term functional outcome (by day 7)	
Favorable	53 (40.2)
No change	47 (36.6)
Unfavorable	21 (15.9)
Death	5 (3.8)
Undetermined	6 (4.5)
Long-term functional outcome (by day 90)	
mRS 0-1	46 (34.8)
mRS 2-5	34 (25.8)
mRS 6 (death)	29 (22.0)
Undetermined	23 (17.4)
Mortality by day 14	18 (13.6)

Intracerebral hemorrhage (ECASS II)	7 (5.3)
aSICH	6 (4.5)
SICH	

mRS, modified Rankin score; ECASS II, European Cooperative Acute Stroke Study II; aSICH, asymptomatic intracranial hemorrhage; SICH, symptomatic intracranial hemorrhage.

II. Laboratory measurements:

The blood samples of 132 patients undergoing thrombolysis were taken on 3 different occasions (before, immediately after and 24h after thrombolysis) and were investigated for FXIII activity, FXIII antigen, TAFI antigen, α2PI activity, α2PI antigen levels. FVIII activity, VWF antigen, D-dimer and fibrinogen levels were also measured. Routine chemistry, hemostasis and hematologic tests were determined from all blood samples. All planned molecular genetic tests (FXIII-A p.Val34Leu, FXIII-A p.Tyr204Phe, FXIII-B p.His95Arg, FXIII-B intronK, α2PI Arg6Trp, FV_{Leiden}, TAFI prom.-438G/A, TAFI p.Ala147Thr, TAFI p.Thr325Ile) were carried out from the DNA samples. In addition to the measurements planned in the Workplan, plasminogen activator inhibitor-1 (PAI-1) activity and antigen levels, and its major polymorphism PAI-1 4G/5G were also determined from the blood samples. A highly researched, relatively new assay, the thrombin generation (TG) test was also carried out in addition. When calculating statistical data regarding TAFI antigen levels and TAFI polymorphisms, we had to realize that that the otherwise high-quality VisuLize TAFI antigen ELISA test (Affinity Biologicals) underestimated TAFI levels in the presence of the TAFI Ile325 allele. As we have found recent literature citing this problem, we had to establish a new TAFI activity test in our laboratory and in the last year of the project we carried out TAFI activity measurements from all (n=396) blood samples.

In a small arm of this study, we investigated the role of the above listed hemostasis factors in the thrombogenic state associated with elevated risk of stroke in a selected population of patients with atrial fibrillation (AF). In this pilot study, 24 patients with AF and 14 controls with other supraventricular tachycardia undergoing transcatheter radiofrequency ablation were included. Blood samples were drawn from the femoral vein (FV), left atrium (LA) and left atrial appendage (LAA) before the ablation procedure. Hemostasis and fibrinolysis factors of interest listed above in this proposal were measured from all samples.

III. Statistical analysis

Results of biochemical and genetic tests were correlated with clinical data. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS 11.5, Chicago, IL) with the following major statistical methods: Shapiro Wilk test of normality, Spearman's/Pearson bivariant correlation, Student's t test/Mann-Whitney U test, paired Student's test/Wilcoxon rank-sum test, ANOVA and Bonferroni post-hoc test or Kruskal-Wallis analysis using Dunn's post-hoc test, for categorical variables contingency tables and χ^2 or Fisher's exact test. When testing for independent risk factors of outcomes, backward binary logistic regression models were used. Adjustment of the models was based on the results of previous statistical analyses, data from literature and methodological principles.

Main findings of the study according to the Research plan and Workplan:

I. The association of FXIII and α_2PI levels and their common polymorphisms with the outcome of thrombolysis in stroke patients

FXIII levels decreased after thrombolysis, particularly in case of more severe stroke. α_2PI levels on admission were significantly lower in patients with NIHSS>5 but were not associated with outcomes. Logistic regression analysis showed that a low FXIII level 24h post-lysis is an independent predictor of mortality. FXIII and α_2PI polymorphisms were without effect. As FXIII levels seemed very high in patients on admission, FXIII levels and polymorphisms were tested in a population control group of 268 healthy individuals. FXIII levels were significantly elevated in patients on admission as compared to this population control group. Findings were presented at 5 international/national congresses. Results on the association of FXIII levels and polymorphisms with stroke thrombolysis outcome were published in 2018 in a well-recognized international journal (Scientific Reports, Nature Publishing Group), the PI was corresponding author (Székely EG, Czuriga-Kovács KR, Bereczky Z, Katona É, Mezei ZA, Nagy A, Tóth NK, Berényi E, Muszbek L, Csiba L, **Bagoly Z**. Low factor XIII levels after intravenous thrombolysis predict short-term mortality in ischemic stroke patients. Sci Rep 2018;8:7662. IF(2017):4.265, PD111929 acknowledged)

Results regarding FXIII levels and polymorphisms in the population control group were also published (Mezei ZA, Katona É, Kállai J, Bereczky Z, Molnár É, Kovács B, Ajzner É, **Bagoly Z**, Miklós T, Muszbek L. Regulation of plasma factor XIII levels in healthy individuals; a major impact by subunit B intronK c.1952+144 C>G polymorphism, Thromb Res, 2016;148:101-106, IF:2.650, PD111929 acknowledged).

Results on α_2PI levels and α_2PI Arg6Trp polymorphism were presented at 3 international and a national congress. A manuscript is currently under preparation from the results (**Bagoly Z,** Baráth B, Szegedi I, Kálmándi R, Csiba L, Katona É. Incorporation of α2-plasmin inhibitor into fibrin clots and its association with the clinical outcome of acute ischemic stroke patients.)

II. The association of TAFI levels and its common polymorphisms with the outcome of thrombolysis in stroke patients

TAFI levels significantly decreased after thrombolytic therapy. Low levels of TAFI on admission were associated with stroke severity. TAFI Thr325Ile polymorphism increased stroke severity (OR: 2.6; 95%CI:1.1-6.6), but did not influence outcomes. Preliminary results on TAFI were presented as an oral presentation at a local congress. Statistical analysis of TAFI antigen levels and polymorphisms were completed in early 2018, however, as additional measurements had to be performed (TAFI activity), results were re-evaluated and the manuscript is currently being prepared (Székely EG, Czuriga-Kovács KR, Nagy A, Berényi E, Csiba L, **Bagoly Z**. Low TAFI levels on admission is associated with stroke severity in ischemic stroke patients within 4.5 hours of their symptom onset.)

III. The association of thrombin generation (TG) parameters with the outcome of thrombolysis in stroke patients

Among TG parameters, Endogenous Thrombin Potential (ETP) and Peak Thrombin were significantly lower in patients with cardioembolic IS. Symptomatic ICH was significantly associated with low ETP and Peak Thrombin levels. It was revealed that an ETP result in the lower quartile is an independent predictor of mortality within 3 months after the event (OR:5.28; 95%CI:1.27-21.86, $p<0.05$). Results were presented at 2 international congresses in 2016/2017, a paper was written and published in Plos

One in 2017, the PI is the corresponding author (Hudák R, Székely EG, Kovács KR, Nagy A, Hofgárt G, Berényi E, Csiba L, Kappelmayer J, **Bagoly Z**. Low thrombin generation predicts poor prognosis in ischemic stroke patients after thrombolysis. Plos One 2017; 12:e0180477, IF: 2.766, PD111929 acknowledged). As thrombin generation is highly affected by antiplatelet agents, the efficacy of antiplatelet therapy was tested in stroke 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)

IV. The association of elevated FVIII activity and VWF antigen levels with the outcome of thrombolysis in stroke patients

VWF levels at all investigated time points and FVIII activity before and 24h after therapy were associated with worse 24h post-lysis ASPECT scores. Elevated FVIII/VWF levels post-lysis were independently associated with poor functional outcomes (mRS>3) at 90 days. Findings were presented at 4 international congresses in 2016/17. A manuscript was written and it was published in Frontiers in Neurology, the PI is corresponding author (Tóth NK, Székely EG, Czuriga-Kovács KR, Sarkady F, Nagy O, Lánczi LI, Berényi E, Fekete K, Fekete I, Csiba L, **Bagoly Z**. Elevated Factor VIII and von Willebrand Factor Levels Predict Unfavorable Outcome in Stroke Patients Treated with Intravenous Thrombolysis. Front Neurol 2018; 8:e721. doi.org/10.3389/fneur.2017.00721. IF(2017): 3.508. PD111929 acknowledged).

In patients with AF, levels of FVIII and VWF were significantly elevated as compared to controls. PAP complex and D-dimer levels were significantly elevated in the LA as compared to FV samples, indicating a temporary thrombotic risk associated with the catheterization procedure. Results were published in 2017, the PI is the corresponding author (Tóth NK, Csanádi Z, Hajas O, Kiss A, Nagy-Baló E, Kovács KB, Sarkady F, Muszbek L, Bereczky Z, Csiba L, **Bagoly Z**: Intracardiac hemostasis and fibrinolysis parameters in patients with atrial fibrillation, Biomed Res Int 2017; 2017:3678017, IF: 2.583, PD111929 grant acknowledged).

V. The association of PAI-1 activity/antigen and PAI-1 4G/5G polymorphism with the outcome of thrombolysis in stroke patients

PAI-1 activity levels dropped transiently after thrombolysis, while PAI-1 antigen levels remained unchanged. PAI-1 4G/5G polymorphism had no effect on PAI-1 levels and did not influence stroke severity. PAI-1 activity/antigen levels on admission were significantly elevated in patients with worse 24h ASPECTS. The presence of 5G allele conferred a significant risk for ICH. A manuscript has been prepared and submitted to Translational Research (Szegedi I, Nagy A, Sarkady F, Székely EG, Czuriga-Kovács KR, Berényi E, Csiba L, **Bagoly Z.** Plasminogen activator inhibitor-1 (PAI-1) 5G/5G genotype is an independent risk factor for intracranial hemorrhage in post-lysis stroke patients.)

The PI published 3 review/editorial papers in 2017/18, which are related to the NKFI PD111929 grant's topic (clot structure, fibrinolysis and recurrent risk of thrombosis). In all 3 papers the help of the PD111929 grant was acknowledged (**Bagoly Z**. Uncovering the genetic background of natural anticoagulant deficiencies: time to look behind the scenes. Pol Arch Int Med 2017; 127:465-467, IF: 2.658, **Bagoly Z**, Ariens RAS, Rijken DC, Pieters M, Wolberg AS. Clot structure and fibrinolysis in thrombosis and hemostasis. Biomed Res Int 2017; 2017:4645137, IF: 2.583 and **Bagoly Z**. Altered fibrin clot phenotype as predictor of the risk of recurrent venous

thromboembolism: evidence is growing. Pol Arch Intern Med 2018; 128:569-571. IF(2017): 2.658)

Other scientific achievements, recognitions related to the project's subject:
In 2016 the PI was invited to be the Lead Guest Editor of a Special Issue on a closely related topic (Clot Structure and Fibrinolysis in Thrombosis and Haemostasis) for Biomed Res Int (IF: 2.583). The Special Issue was published in 2017. During the PD111929 project, the work of the PI was recognized as she was appointed to a number of leading positions in international/national societies: she was re-elected as a Co-Chair of the International Society on Thrombosis and Haemostasis (ISTH), FXIII and Fibrinogen Scientific and Standardization Committee (SSC) for 2016-19. She was appointed to be an Advisory Board Member of the European Hematology Association (EHA) Scientific Program Committee (SPC) for 2017-19. In 2018, she was elected an International Fibrinogen Research Society Council Member-Class of 2024 (for the period of 2018-2024). She was elected the National Representative and Executive Board Member of the Hungarian Society of Laboratory Medicine (2018-20). In 2017 she became a National Excellence Research Program Awardee and also received the Lajos Szodoray Prize of the University of Debrecen. In 2018 she was elected an Executive Board Member of the Hungarian Society of Thrombosis and Haemostasis (2018-20). With the help of the project, a PhD student supervised by the PI has finished her thesis, the local defense process in October 2018 was successful and the public defense is expected in December 2018.