EARLY NEUROLOGICAL IMPROVEMENT FOLLOWING REPERFUSION THERAPY IN ACUTE ISCHAEMIC STROKE

PhD THESIS BOOKLET

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Szeged
2023

1. Introduction

According to the recent Global Burden of Disease estimates, stroke is the second leading cause of death and the third leading cause of combined death and disability worldwide. The lifetime risk of stroke has increased by approximately 50% in the last 20 years, and affects one in four people. Ischaemic events account for the majority of stroke cases.

Acute treatment of ischaemic stroke has developed significantly in recent years. Intravenous thrombolysis (IVT) with alteplase or tenecteplase (recombinant tissue-plasminogen activators) is the first-line treatment in patients who present within 4.5 hours of symptom onset. In selected cases, the time window for treatment can be extended to 9 hours. For patients with large-vessel occlusion (LVO), mechanical thrombectomy (MT) is an effective endovascular procedure for recanalization. Meta-analyses indicate a number needed to treat value of 2.6 to reduce disability in patients with anterior circulation LVO. In selected cases, MT can be performed up to 24 hours after symptom onset.

Most randomized clinical trials in ischaemic stroke assess the efficacy of treatment or intervention with the modified Rankin Scale (mRS). The mRS is a 7-point scale (from 0 to 6) that indicates the degree of disability in patients' daily activities, with an emphasis on mobility. Higher scores indicate more severe disability. It is usually measured approximately three months after stroke. The mRS is brief, simple and has high inter-rater reliability. However, the mRS does not account for comorbidities, polypharmacy, socioeconomic factors, availability and quality of rehabilitation, and support provided by family.

Therefore, we believe that the treatment effect of reperfusion therapies may be better reflected by short-term changes in the National Institutes of Health Stroke Scale (NIHSS) score rather than the 90-day mRS score. The NIHSS measures impairment rather than disability, with scores ranging from 0 to 42, with higher scores indicating a more severe deficit. The short-term improvement in NIHSS score is usually termed early neurological improvement (ENI). Unfortunately, the definition of ENI varies significantly among studies. Despite the various definitions of ENI, several studies have shown that ENI correlates with clinical outcomes at later time points.

The thesis will focus on ENI after reperfusion therapies (IVT and MT). The definition of ENI varied among the studies included in this thesis. We aimed to tailor the definition of ENI to suit the investigated stroke population and to best correlate with functional outcomes.

1.1. DWI-FLAIR mismatch guided thrombolysis

The determination of the exact time of symptom onset in acute ischaemic stroke is often difficult. Approximately 14-27% of stroke patients present to the emergency department with unknown symptom onset time. Current guidelines recommend systemic IVT within 4.5 hours after symptom onset. If the onset time is unknown and the patient was last seen well more than 4.5 hours ago, specific imaging modalities can help to establish eligibility for thrombolytic therapy. One option is the MRI-based diffusion-weighted imaging (DWI) – fluid-attenuated inversion recovery (FLAIR) mismatch concept. DWI detects ischaemia-induced changes in cellular water diffusion within minutes. In comparison, T2 weighted imaging and, consequently, FLAIR imaging are sensitive to only detect net water increase and vasogenic oedema that follows cytotoxic oedema. In a multicentre, randomised, double-blind, placebo-controlled trial (Efficacy and Safety of MRI-based Thrombolysis in Wake-up Stroke, i.e. the WAKE-UP trial), alteplase treatment was administered for patients with unknown stroke onset times who were last seen well more than 4.5 hours before symptoms were noticed, and had DWI-FLAIR mismatch. Patients treated with alteplase had significantly better functional outcomes at 90 days than those who received placebo.

1.2. Prognostic biomarkers in acute ischaemic stroke

Biomarkers are objective indicators of physiological or pathological processes and have valuable applications in predicting and monitoring clinical responses to therapeutic interventions. Despite a significant increase in the number of blood-based biomarkers reported, prognostic biomarkers that could aid in predicting the outcome of ischaemic stroke or response to reperfusion therapy are still lacking in routine clinical practice. Although it's prognostic value has not been elaborated yet, animal models and clinical studies have unequivocally proved that the kynurenine (KYN) pathway is activated in acute ischemic stroke.

1.3. Kynurenine pathway

The KYN pathway is the main route of tryptophan (TRP) metabolism. It plays a vital role in N-methyl-D-aspartate (NMDA) receptor (NMDAR) mediated excitotoxicity, reactive oxygen species (ROS) production and inflammation. The first and rate-limiting step is the metabolism of TRP to L-KYN by indoleamine-2,3-dioxygenase (IDO) or tryptophan-2,3-

dioxygenase (TDO). L-KYN can be further metabolised into three distinct molecules. Kynurenine aminotransferase (KAT), kynurenine-3-monooxygenase (KMO), and kynureninase enzymes catalyse the production of kynurenic acid (KYNA), 3-hydroxy-L-kynurenine (3-HK) and anthranilic acid (AA), respectively. 3-HK and AA can be metabolised to 3-hydroxyanthranilic acid (3-HAA) and quinolinic acid (QUIN). The molecules mentioned above are collectively called kynurenines. The most significant neuroactive compounds of the KYN pathway are KYNA, 3-HK and QUIN. KYNA is a known endogenous, competitive inhibitor of NMDAR. 3-HK is a neurotoxic compound that mediates the production of free radicals. QUIN is also a neurotoxic metabolite, which acts as a potent NMDAR agonist and leads to glutamatergic excitotoxicity.

1.4. Near-infrared spectroscopy (NIRS)

Monitoring acute ischaemic stroke patients during reperfusion therapies (IVT or MT) is based mainly on frequent neurological and physical examinations. Near-infrared spectroscopy (NIRS) is a bedside, noninvasive, continuous, real-time tool that can be used to monitor acute stroke patients. To our knowledge, only a few observational and pilot studies have reported the potential of NIRS monitoring during acute ischaemic stroke.

NIRS utilizes a light source that emits photons in the near-infrared range (700-1100 nm). These photons penetrate the skull a few centimetres deep into the brain parenchyma. The emitted light is partly redirected, scattered and absorbed. The absorption spectrum of oxyhaemoglobin (Hb_{oxy}) and deoxyhaemoglobin ($Hb_{deoxy)}$ varies at different wavelengths. This difference allows for the calculation of Hb_{oxy} and Hb_{deoxy} concentrations based on the difference in intensity of emitted and received light, using the Beer-Lambert equation:

$$A = \lg\frac{I_0}{I} = \varepsilon \times c \times l$$

(A: absorption, I_0: intensity of emitted light, I: intensity of received light, ε: absorption coefficient, c: concentration, l: photon pathlength).

Total haemoglobin (Hb_T) concentration is the sum of Hb_{oxy} and Hb_{deoxy} concentrations and is proportional to cerebral blood volume (CBV). Therefore, NIRS can measure cortical blood oxygenation/saturation, i.e. the fraction of Hb_{oxy} relative to Hb_T.

It is well known that the collateral circulation plays a pivotal role in reducing the progression of ischaemic brain damage. However, a real-time assessment tool of collateral circulation in acute ischaemic stroke is lacking. Multimodal MRI studies have shown that augmented CBV, preserved cerebral blood flow (CBF) and delayed mean transit time implies the presence of collateral flow. Taussky et al. used CT perfusion to demonstrate a linear correlation between regional O_2 saturation (rSO_2) and CBF. Therefore, both CBV and CBF correlate with rSO_2 values, which can be measured in real-time at the bedside, non-invasively with NIRS.

2. Aims

The aims of this thesis were:

I. Analyse ENI after alteplase treatment in patients presenting with DWI-FLAIR mismatch. Furthermore, we compared treated patients' outcomes to those not eligible for IVT due to no DWI-FLAIR mismatch or other contraindications. Our analysis included patients with unknown symptom onset times and cases where MRI was performed because of diagnostic uncertainty within 4.5 hours after symptom onset. Patients with LVO were excluded.

II. Perform a pilot study to investigate whether metabolites of the KYN pathway and activity of relevant enzymes measured before and 12 hours after IVT could be potential biomarkers for predicting ENI.

III. Explore if NIRS is suitable for monitoring anterior watershed territory leptomeningeal collateral circulation, and analyse the correlation between NIRS readings (during IVT and MT) and ENI.

3. Methods

3.1. Patients

3.1.1. *Patients in the DWI-FLAIR mismatch guided thrombolysis study*

A retrospective single-centre observational study was conducted. We identified patients between January 2017 and April 2020, with a suspected clinical diagnosis of acute ischaemic stroke, where an MRI showed DWI hyperintensity. Patients with LVO were excluded. We used a more permissive protocol than the WAKE-UP trial: patients with partial

DWI-FLAIR mismatch were also eligible for treatment, and pre-stroke functional dependence or age were not contraindications for IVT. Patients who received alteplase had repeat imaging (CT or MRI) approximately 24 hours after IVT. All patients or their legal representatives gave informed consent before treatment. The Ethics Committee of the University of Szeged, Albert Szent-Györgyi Health Centre, approved our study (ID: 6/2017-SZTE), which was conducted according to the revised Declaration of Helsinki. Parameters of patients treated with alteplase were compared to those who did not receive this treatment due to a matched DWI-FLAIR pattern or other contraindications.

3.1.2. Patients in the biomarker pilot study

Our study on prognostic biomarkers included acute ischemic stroke patients who underwent IVT with alteplase between January and December 2018. We excluded patients who had MT and those with a baseline mRS score >2. Patient data were collected from the Stay Alive Acute Stroke Registry, a national, multicentric database. All patients or their legal representatives gave informed written consent before storing their data in the Registry. The biomarker pilot study was conducted in accordance with the revised Declaration of Helsinki, and the Ethics Committee of the University of Szeged, Albert Szent-Györgyi Clinical Centre approved the protocol (GINOP 2.3.2-15- 2016-00048). All patients or their relatives gave informed consent for inclusion before participation in the biomarker study.

3.2. Imaging protocol in the DWI-FLAIR mismatch guided thrombolysis study

Each patient underwent an acute multimodal brain MRI with a 1.5 T GE Signa Excite MRI scanner. The acute stroke MRI protocol included DWI, FLAIR, and susceptibility-weighted angiography (SWAN). DWI-FLAIR mismatch was defined as an ischaemic DWI lesion with no corresponding signal change on the FLAIR sequences. Partial mismatch was defined as a corresponding FLAIR signal change more diminutive than the DWI hyperintensity. The attending radiologist and neurologist rated the mismatch patterns visually.

3.3. Sampling in the biomarker pilot study

Peripheral venous blood samples for the biomarker study were taken just before IVT, and 12 hours after the initiation of treatment (samples A and B, respectively). Blood samples were centrifuged at 3000/min for 13 min, and sera were stored at -80 °C until further analysis.

Measurement of KYN metabolites was done by ultra-high-performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS).

3.4. Methodology of the NIRS pilot study

The Ethics Committee of the University of Szeged, Albert Szent-Györgyi Clinical Centre approved our study (ID: 211/2016-SZTE). All participating patients or first-degree relatives gave written informed consent prior to NIRS monitoring. We used the INVOS™ 5100C Cerebral/Somatic Oximeter (Medtronic, Minneapolis, MN, USA) for 24-hour continuous monitoring. The NIRS sensors were placed over bilateral frontal areas corresponding to the anterior watershed territories. Baseline rSO_2 was measured before IVT was started. rSO_2 measurements were made approximately every 30 seconds. We analysed the 5-minute average rSO_2 values registered at the start of IVT and at 1 hour, 6 hours, 12 hours, 18 hours and 24 hours after the initiation of treatment. Interhemispheric rSO_2 difference ($IH\Delta rSO_2$) was calculated as rSO_2 on the affected side minus rSO_2 measured above the contralateral side. Based on previous articles, a 4% change in rSO_2 value and a 2% change in $IH\Delta rSO_2$ were considered significant. If LVO was present, collateral circulation on imaging was determined by a neuroradiologist using a 3-grade scale (good-intermediate-poor).

3.5. Outcome measures

3.5.1. Outcome measures in the DWI-FLAIR mismatch guided thrombolysis study

We analysed baseline and discharge NIHSS scores as outcome of efficacy. We defined ENI as any neurological improvement, as indicated by a lower NIHSS score at discharge compared to baseline. Regarding safety outcomes, we analysed the occurrence of intracerebral haemorrhage (ICH) on 24-hour repeat imaging after IVT. The extent of the haemorrhage was graded according to the European Cooperative Acute Stroke Study II (ECASS II). Symptomatic ICH (sICH) was also defined according to the ECASS II trial criteria.

3.5.2. Outcome measures in the biomarker pilot study

Efficacy endpoints in our biomarker study were ENI and good functional outcome at 30 and 90 days after the stroke. ENI was defined according to the Neurological Disorders and Stroke Recombinant Tissue Plasminogen Activator Stroke Study (NINDS) criteria: at least a 4-point decrease in the NIHSS score or the patient became symptom free. However, contrary

to the NINDS trial, we used the NIHSS score at discharge, not at 24 hours after treatment. The criterion for good functional outcome was an mRS score ≤ 2.

3.5.3. Outcome measures in the NIRS pilot study

Clinical outcomes were assessed with NIHSS and mRS scores. ENI was defined as per the NINDS criteria at 24 hours. Good functional outcome was an mRS score ≤ 2 at three months.

3.6. Statistical analysis

ENI and good outcomes at 30 and 90 days were categorical variables in the DWI-FLAIR mismatch guided thrombolysis and biomarker studies.

Continuous clinical variables were expressed as mean ± SD in the biomarker pilot study. The distribution of KYN metabolites and enzymatic activities were analysed with the Shapiro-Wilk test. Normally distributed variables were expressed as mean ± SD. Non-parametric data were expressed as median and IQR. Pre-thrombolysis and 12-hour KYN metabolite concentrations and enzymatic activities were compared with either paired sample t-test or Wilcoxon matched-pairs signed-ranks test (depending on data distribution). To compare means of concentrations and enzymatic activities between groups with and without ENI or good functional outcome, we used the independent sample t-test or Mann-Whitney U test (depending on the distribution of data). Boxplots were drawn to allow for better visualisation of statistically significant findings. Furthermore, we performed receiver operating characteristic (ROC) analysis if statistical significance was met. We calculated area under the curve (AUC), as well as sensitivity (SN) and specificity (SP) for different cut-off values. Logistic regression was not performed due to the small sample size.

In the DWI-FLAIR mismatch guided thrombolysis study, the Shapiro-Wilk test was applied to analyse the distribution of continuous variables. Parametric data were expressed as mean ± SD, whereas non-parametric data were expressed as median and IQR. Between-group comparisons were made with independent samples t-test. Pearson's chi-squared test of independence was applied to compare categorical variables. Fisher's exact test was used where sample sizes were small (i.e. equal to or less than 5). To compare ENI and mortality between the groups, 95% confidence intervals (CI) and odds ratios (OR) were calculated by

standard approaches. We also performed multivariable logistic regression to analyse the correlation between thrombolysis and ENI. We applied a backward likelihood ratio model selection method. Variables included in the analysis were age, gender, admission blood glucose, admission systolic and diastolic blood pressure, baseline NIHSS and thrombolysis. OR and 95% CI were again calculated.

In both studies, a p-value of <0.05 was regarded as statistically significant. 95% confidence intervals (CI) were presented where appropriate. All analyses were carried out with IBM SPSS (version 22, IBM Corp., Armonk, USA) statistical software.

Due to the small sample size of the NIRS pilot study, we did not draw statistical conclusions. Instead, we set out to describe the five NIRS recordings, and explain how these findings could correlate with cerebrovascular haemodynamics and clinical outcome.

4. Results

4.1. Results of the DWI-FLAIR mismatch guided thrombolysis study

We identified 121 patients with a clinical diagnosis of acute ischaemic stroke and DWI hyperintensity on their MRI. After exclusion of patients for various clinical and imaging reasons, 71 patients were included in our final analysis.

Twenty-nine patients received IVT. Six patients had partial DWI-FLAIR mismatch on MRI. Two of these patients were not thrombolysed due to sulcal siderosis in one and a previous intracerebral haemorrhage in the other case, as detected on SWAN. Four patients had DWI-FLAIR mismatch but did not receive alteplase due to contraindications We detected significantly more lacunar infarcts in non-thrombolysed patients (p=0.042). Otherwise, the two groups were well balanced.

4.1.1. Efficacy and safety outcomes

Significantly more patients had ENI in the thrombolysed group (OR, 3.16; 95% CI, 1.178-8.479; p=0.020). In the multivariable logistic regression analysis, IVT was the only variable correlated with ENI (OR, 3.051; 95% CI, 1.135-8.206; p=0.027).

Only patients in the thrombolysis arm had follow-up imaging. There were no clinical indications for a repeat scan in any non-thrombolysed patients. Two thrombolysed patients

had ICH on follow-up imaging (6.90%). One patient had parenchymal haemorrhage type I (PH 1), and another had a remote PH 2. Only the patient with PH 2 had an sICH (3.45%). He died five days after IVT.

Eighteen patients were lost to long-term follow-up. Of the remaining 53 individuals, six died within 90 days after stroke (11.32%): two in the thrombolysed (n=20, 10.00%) and four in the non-thrombolysed group (n=33, 12.12%), with statistically non-significant odds (OR, 0.81, 95% CI, 0.134-4.856; p=1.000).

4.2. Results of the biomarker pilot study

Our pilot study included 48 patients. Thirty-nine were known to be within the 4.5-hour thrombolysis time window. In the remaining nine patients with unknown stroke onset time, IVT was carried out based on DWI-FLAIR mismatch on acute brain MRI, as per the WAKE-UP trial.

Significant changes in paired serum levels were observed for KYN, AA, KYNA, xanthurenic acid (XA), picolinic acid (PICA) and QUIN. Enzymatic activity of IDO, monoamine oxidase (MAO) and KAT was calculated by the following ratios: KYN/TRP, 5 hydroxy-3-indoleacetic acid (5-HIAA)/serotonin (SERO) and KYNA/KYN, respectively.

Patients with ENI had significantly lower concentrations of KYNA and lower KAT activity in sample A (independent sample t-test, p=0.01, and Mann-Whitney U test, p=0.002, respectively, **Figure 1**).

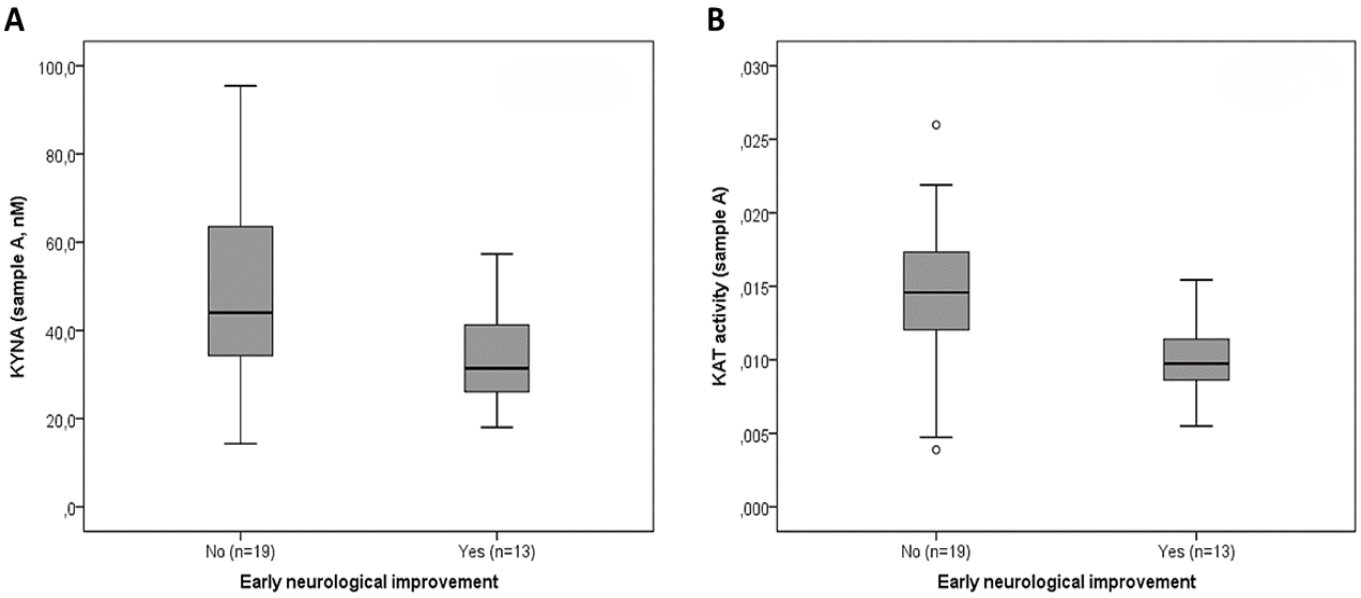


Figure 1. Boxplots highlight the significant difference between pre-treatment KYNA levels (A) and KAT activity (B) between patients without and with ENI. For each box, the horizontal line inside the box shows the median. The ends of the boxes represent the first and third quartiles. The whiskers extend to the highest and lowest values not considered outliers (defined as 1.5 times the IQR). Outliers are shown as circles.

We performed ROC analysis for ENI with KYNA levels and KAT activity measured before treatment (**Figure 2**). AUC for KYNA concentrations was 0.74, 95% CI 0.57-0.91, p=0.02. The optimal cut-off concentration to predict ENI was 37.8 nM (SN 69.2%, SP 68.4%). Similarly, AUC for KAT activity was 0.82, 95% CI 0.67-0.98, p=0.002, and the optimal cut-off activity was 0.0127 (SN 92.3%, SP 73.7%).

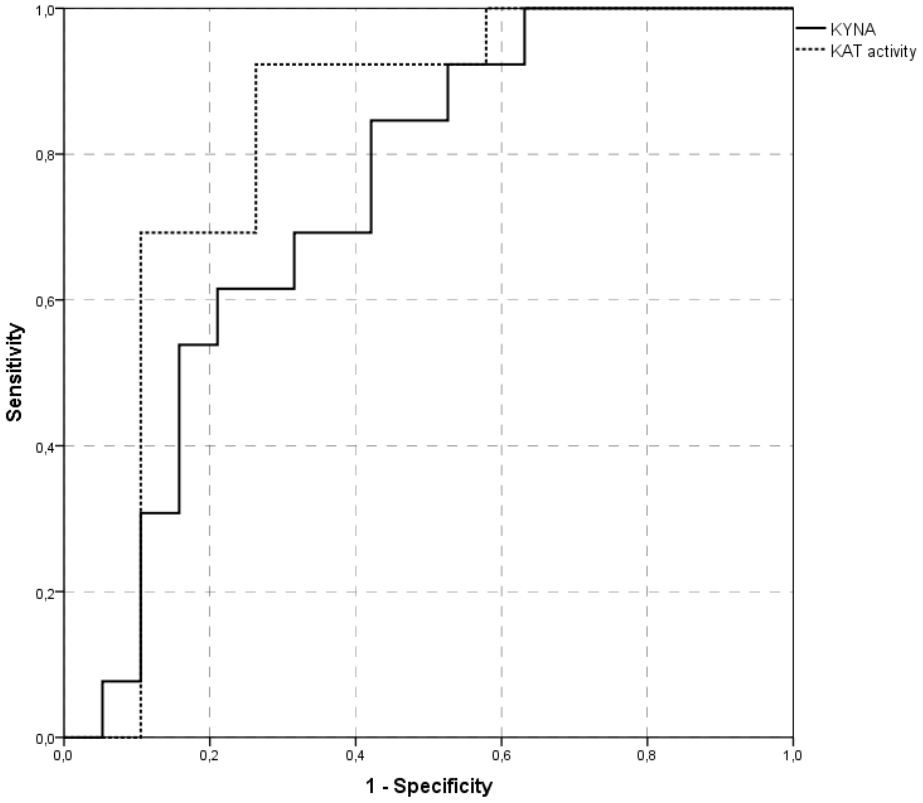


Figure 2. ROC curve showing the accuracy of pre-thrombolysis KYNA concentration and KAT activity in predicting ENI.

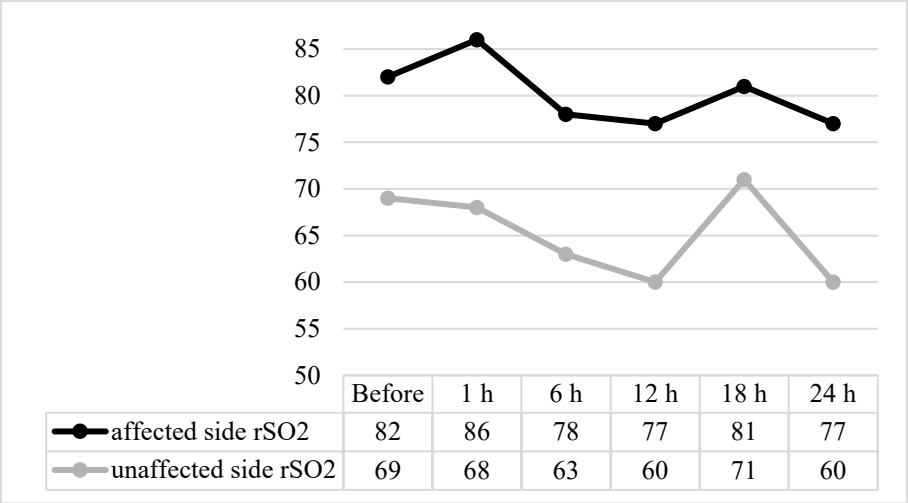
4.3. Results of the NIRS pilot study

Our study population included five acute stroke patients with left-sided anterior circulation infarcts. Patient 3 had MT due to M1 segment occlusion. All participants received

alteplase according to the Hungarian Acute Ischaemic Stroke Diagnostic and Treatment Recommendations. Blood pressure, SpO_2, heart rate and electrocardiography were monitored for all patients throughout the study period. In the thesis booklet, only the detailed description of Patient 5 is presented.

4.3.1. Descriptive analysis of the NIRS recording of Patient 5

Patient 5 had elevated rSO_2 values above the ipsilateral hemisphere (82% vs 69%). The significantly high $IH\Delta rSO_2$ was possibly a consequence of chronic right ICA occlusion, leading to collateralization via the circle of Willis, and consequent enlargement of left ICA, MCA, and anterior cerebral artery (ACA). Increased blood flow in the left MCA and ACA explains the high rSO_2 values above the ipsilateral watershed area, implying well-developed leptomeningeal collaterals. The $IH\Delta rSO_2$ value remained high throughout the 24-hour monitoring, and the patient achieved ENI and good functional outcome at three months.



	Before	1 h	6 h	12 h	18 h	24 h
affected side rSO2	82	86	78	77	81	77
unaffected side rSO2	69	68	63	60	71	60

Figure 3. rSO_2 (%) trends of Patient 5.

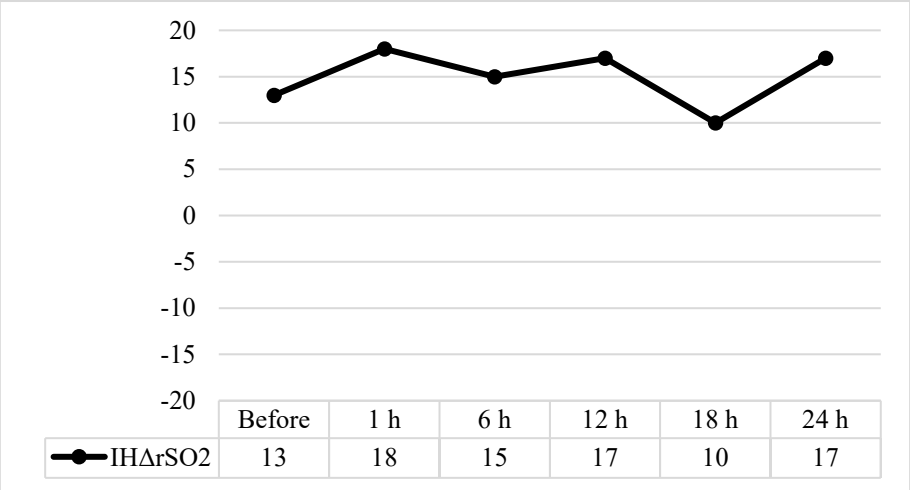


Figure 4. IHΔrSO$_2$ (%) trend of Patient 5.

5. Discussion

5.1. Discussion of the DWI-FLAIR mismatch guided thrombolysis study

Our real-world data support using the DWI-FLAIR mismatch concept for alteplase treatment in acute ischaemic stroke patients without LVO. We analysed ENI to investigate the short-term response to alteplase because the beneficial effect of IVT could be better reflected by the change in NIHSS score rather than the 90-day mRS score. As the baseline NIHSS scores were relatively low in our study (a median of 5 and 4.5 points in the thrombolysed and non-thrombolysed arms, respectively), we felt that defining ENI similar to the NINDS trial was not practical. We measured NIHSS scores at discharge, which in our opinion, is reasonable practice considering that the median length of hospital stay was only five days in both groups.

Due to the relatively low baseline NIHSS scores, we felt that any short-term improvement in the NIHSS could be considered a favourable response to treatment that would lead to a good functional outcome at 90 days. Unfortunately, 90-day outcome data was scarce. Consequently, we could not correlate our definition of ENI with 90-day mRS scores.

Regarding safety outcomes, we compared our results with those of the WAKE-UP trial. The percentage of PH2 according to ECASS II was similar: 3.45% in our study compared to 4.0% in the randomised clinical trial. The percentage of sICH was also similar: 3.45% in our population vs 2.8% in the WAKE-UP trial.

In patients who received alteplase, the 10% mortality at 90 days in our population was higher than the 4.1% in the WAKE-UP study. Potential explanations are that our patients

were older with more vascular risk factors, and were more disabled at baseline. We did not contraindicate alteplase treatment based on mRS, whereas only patients with mRS 0-1 were included in the WAKE-UP trial.

Our study included only four patients who had thrombolysis with DWI-FLAIR partial mismatch. Therefore, meaningful conclusions about the safety and efficacy of alteplase treatment in patients with such imaging patterns cannot be drawn.

Limitations of our study include the small sample size and the retrospective observational nature of data collection. Based on their DWI-FLAIR pattern, the non-thrombolysed group comprised patients with onset times most probably beyond 4.5 hours. In addition to comparing the rates of ENI between the two groups, we also performed a logistic regression analysis to investigate the predictors of ENI. This regression analysis identified IVT as the only variable associated with ENI.

5.2. Discussion of the biomarker pilot study

To our knowledge, we performed the first study that analysed changes in KYN metabolite serum levels and enzymatic activities in acute ischemic stroke patients who received IVT. The main finding of our pilot study is that patients with ENI have significantly lower concentrations of KYNA and lower KAT activity before alteplase treatment. We therefore propose that pre-thrombolysis KYNA levels and KAT activity are potential biomarkers of ENI.

It should be highlighted that similarly to the DWI-FLAIR mismatch guided thrombolysis study, ENI was assessed based on the difference between the admission and discharge NIHSS scores (24-hour NIHSS scores were unavailable). Since it suited our population, we applied the NINDS criteria to define ENI (mean baseline NIHSS score was 8.81). About 40% of patients achieved ENI, and 60% had a favourable outcome at 30 and 90 days after IVT. In univariate analysis, patients with ENI had significantly higher odds of achieving mRS ≤ 2 at 30 days (OR 5.385, 95%CI 1.261-22.987, p=0.023) compared to patients who did not show ENI. The same was true for 90-day functional outcomes, but this trend did not meet statistical significance in the univariate model (OR 4.333, 95%CI 0.978-19.202, p=0.054).

It is important to note that KYN metabolites were measured from the serum of patients. Therefore, our findings do not reflect the intracerebral changes in KYN metabolites and enzymes in acute ischemic stroke.

5.3. Discussion of the NIRS pilot study

Our NIRS monitoring study investigated whether NIRS is feasible to evaluate leptomeningeal collaterals at the anterior watershed areas and analyse the correlation between NIRS readings and ENI. Although our findings are exploratory because of the small sample size, we believe an excellent example is the case of Patient 5. Due to a chronic right ICA occlusion, significantly higher rSO_2 values were measured above the left hemisphere. A possible explanation for this finding is an increased blood flow in the left carotid system, which provides adequate perfusion to both hemispheres through the circle of Willis. Long-standing increase of blood flow has led to the enlargement of these vessels and a well-developed leptomeningeal collateral circulation. The collateral circulation remained stable (the IHΔrSO_2 was stable and high throughout the 24-hour monitoring), and the patient achieved ENI and good functional outcome at three months.

According to the NINDS criteria, three out of five patients achieved ENI at 24 hours. At three months, four patients had favourable functional outcomes. If we apply the NINDS criteria for the discharge NIHSS scores, all four patients with mRS ≤ 2 at three months had ENI.

NIRS parameters could guide clinicians in finding individually tailored target blood pressure and SpO_2 ranges. For instance, patients with acute ICA occlusion could benefit from increased systemic blood pressure to maintain adequate collateral circulation until recanalization. A pre-clinical study investigated this concept and found that mild induced hypertension increased cortical collateral blood flow and significantly reduced infarct volume in mice with transient distal MCA occlusion.

We performed NIRS monitoring with two channels placed over the two frontal areas. It would provide additional information if more sensors were placed over the cerebral hemispheres. Rummel et al. used multichannel NIRS monitoring during transient balloon occlusion of cerebral arteries, and demonstrated different rSO_2 changes over the ischaemic core and watershed areas due to haemodynamic changes in collateral flow.

5.4. Proposal for a new criterion of early neurological improvement

There are various definitions for ENI after reperfusion therapy in the literature. Despite the different definitions, ENI correlates well with long-term functional outcomes measured with the mRS. The actual treatment effect of IVT and MT is probably best reflected in the 24-hour change in the NIHSS score. Although discharge NIHSS scores have been shown to correlate with functional outcomes at three months similarly, it is also dependent on other therapeutic interventions and preventive measures.

How the change in NIHSS scores should be measured is still controversial. **Table 7** proposes new criteria to define ENI, based on previous studies and clinical experience. We categorise patients into three groups based on their initial NIHSS score. In the Study of the Efficacy and Safety of Alteplase in Participants With Mild Stroke (PRISMS) trial, approximately 90% of patients with minor, non-disabling strokes (defined as NIHSS score of 0 to 5, and the deficits were judged not to be clearly disabling) had an mRS 0-2 at 90 days. The percentage change in the criterion was based on the work of Agarwal et al.

Stroke severity	Definition for ENI
Minor stroke (NIHSS ≤ 5)	at least 1 point decrease in NIHSS score at 24 hours
Moderate stroke (5 < NIHSS < 15)	NIHSS becomes ≤ 5 (i.e. minor stroke) or at least 40% improvement in NIHSS score compared to baseline at 24 hours
Severe stroke (16 ≤ NIHSS)	at least 40% improvement in NIHSS score compared to baseline at 24 hours

Table 7. Proposed new criteria for ENI

6. Conclusions

Investigation of ENI is essential in predicting prognosis after IVT and MT. Good collateral circulation is pivotal in reducing the progression of ischaemic brain damage and achieving ENI. Based on our work, NIRS monitoring provides valuable real-time information on the state of leptomeningeal collaterals, and helps to assess the effects of reperfusion therapies.

The thesis contains the first study that has analysed the changes in KYN metabolite serum levels and enzymatic activities in acute ischemic stroke patients who received IVT. We propose that baseline serum KYNA concentration and KAT activity are potential biomarkers of ENI.

Furthermore, the thesis provides real-world data on the association between ENI and IVT in acute ischaemic stroke patients with a DWI-FLAIR mismatch in the absence of LVO. The rate of haemorrhagic complications was similar to those published in large clinical thrombolysis trials with known onset times.

The definition of ENI varied among the studies included in this thesis. To avoid future controversies, new criteria for ENI have been proposed based on previous definitions and clinical expertise. ENI established using our proposed new criteria could serve as a helpful efficacy outcome measure for future clinical trials assessing new treatments for acute stroke.

7. Acknowledgements

I am grateful for the academic and personal support and contribution of my supervisors, Péter Klivényi and László Sztriha. I am indebted to László Vécsei for allowing me to work at the Department of Neurology, Albert Szent-Györgyi Clinical Centre, University of Szeged. I would also like to thank the help of all the colleagues who participated in the projects that formed the foundations of this thesis. I am also thankful for the dedicated work of the nurses of the Stroke Unit at the Department of Neurology, Albert Szent-Györgyi Clinical Centre, University of Szeged. I also greatly value the help of my co-workers in the Biobank facility, who handled and stored the blood samples for the biomarker study.
Last but not least, I am incredibly grateful to my family, especially my wife, Bea, for her continued support.

The biomarker and NIRS studies were supported by the project GINOP (2.3.2-15-2016-00048.). The DWI-FLAIR mismatch guided thrombolysis study was supported by the project TKP2020 NKFIH-1279-2/2020 and the University of Szeged Open Access Fund (grant number 5833). The National Talent Program (NTP-NFTÖ-21-B-0206) also supported my work. I am grateful for the help of the Department of Anaesthesiology and Intensive Therapy, Albert Szent-Györgyi Clinical Centre, University of Szeged, for allowing me to use the INVOS™ 5100C Cerebral/Somatic Oximeter for the NIRS study.

For references to the statements in the thesis booklet, see the dissertation.

Publications directly related to the thesis

I. **Annus Á**, Gera FZ, Sztriha L, Klivényi P. DWI-FLAIR mismatch guided thrombolysis in patients without large-vessel occlusion: real-world data from a comprehensive stroke centre. *Heliyon.* 2022 Dec 5;8(12):e12069. DOI: 10.1016/j.heliyon.2022.e12069. (IF: 3.776, [2021])

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Total Impact Factor of original papers directly related to the thesis: **7.299**

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