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Neuron-specific enolase and tau protein as neurobiochemical markers of neuronal damage are related to early clinical course and long-term outcome in acute ischemic stroke

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Abstract

Objectives: Analyses of neuron-specific enolase (NSE) and tau protein in patients with hyperacute ischemic stroke, their association with infarct volume, severity of the neurological deficit, the neurovascular status and functional outcome.
Patients and methods: In 66 consecutive patients, serial venous blood samples were taken at 3, 6, 12, 18, 24, 48, 72, 96, and 120 h after stroke onset. The neurovascular status was assessed by repetitive extra- and transcranial duplex sonography. Neurological deficits were quantified by the NIH stroke scale, and functional outcome was assessed with the modified Rankin scale (mRS).
Results: After a first rise within 3 h, NSE decreased followed by a secondary increase until Day 5. Tau protein concentrations showed a continuous increase from admission onward. NSE and tau release were highly correlated with severity of neurological deficits and infarct volume ($P = 0.001$). NSE, but not tau protein, release was associated to the neurovascular status on admission. NSE and tau protein values were significantly correlated with the functional outcome at 3 months ($P < 0.001$).
Conclusion: Release kinetics of NSE and tau protein are associated with patients' clinical deficits and infarct volume, and may be used as an additional predictor of the early course and functional outcome.
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Keywords: NSE; Tau-protein; Stroke; Outcome

1. Introduction

Neurobiochemical markers of brain damage gained particular attention in patients with vascular and traumatic brain injury during the last decade. Due to commercial availability, protein S100B and neuron-specific enolase (NSE) were most often studied. In patients with ischemic stroke, a number of studies showed a significant association between clinical deficit, infarct volume, functional disability, and the release of protein S100B, indicating that serum levels may add information to stroke course and short-term outcome [1–5]. Furthermore, it has been shown recently, that the site of vessel occlusion [6] and the time of recanalisation [7] highly influence brain damage and are related to different release kinetics of S100B. In addition, protein S100B concentrations 48–72 h after stroke have been introduced as a surrogate marker of the long-term outcome 90 days after stroke [6,8] and were even suggested as a possible endpoint in acute stroke trials [8].

Whereas protein S100B is a glial-derived protein, NSE is a dimeric isoenzyme of the glycolytic enzyme enolase and derives from neuronal cytoplasm and neuroendocrine cells [9]. Several studies have demonstrated significantly lower NSE concentrations in controls than in patients with acute ischemic stroke [2,3,10,11]. However, correlations between NSE values and infarction volume, various stroke scale scores and outcome parameters have been found inconsistent [2,3,5,10–13] Therefore, the association of NSE release with severity of acute symptoms and functional outcome has

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Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1017
000001

been questioned in a recently published review by Anand and Stead [14]. One reason might be that measurements of NSE serum concentrations were based on various techniques, such as in-house RIA, commercially available ELISA or LIA with different detection thresholds, sensitivity and specificity. None of the previous investigations had used a fully automated high-end technique. Besides, a variety of different scales have been applied for the assessment of clinical deficits and functional outcome and some of these (e.g. Glasgow Coma Scale and Outcome Scale) inadequately represent stroke patient's deficits.

Tau protein is a structural microtubule-associated protein that is mainly expressed in neurons and axons. In cerebrospinal fluid (CSF), increased levels of tau protein have been described in neurodegenerative diseases, e.g. Alzheimer's and Creutzfeldt–Jakob disease [15], traumatic brain injury [16], in cerebral complications after aortic surgery [17] and in acute ischemic stroke [18]. Serum analyses of tau protein in acute ischemic stroke were first and so far only performed by Bitsch et al. [19] who described elevated tau levels in 35% of their 30 patients with peak concentrations within 3–5 days. Even though a correlation of peak tau values with infarction volume and area under curve of tau protein and functional disability after 3 months was found, they failed to show a significant association with the severity of clinical deficits. Furthermore, although an investigation of the neurovascular status was not conducted, the rise of tau protein was hypothesized to indicate reperfusion [19].

To overcome the noted limitations of previous NSE studies [14] and to answer the question whether tau protein is a marker of reperfusion, we investigated the relation between release patterns and serum concentrations of NSE and tau protein as neuronal marker proteins in patients with acute ischemic stroke, their association with infarct volume, severity of the neurological deficit, the neurovascular status and patient's functional outcome.

2. Subjects and methods

Sixty-six consecutive patients above 18 years of age admitted to the stroke unit of our department of neurology within 6 h (median 3 h) after the onset of first symptoms of acute stroke of the middle cerebral artery were investigated. Local ethics committee had approved the study.

After patient's informed consent, serial venous blood samples were obtained hourly between 1 and 6 h, at 12, 18, 24, 48, 72, 96, and 120 h after stroke onset for repetitive measurements of serum levels. Blood was allowed to clot, and after centrifugation (1000 *g*, 10 min) within 30 min, serum was stored at −78 °C for later analysis. Tau protein concentrations were measured by a commercially available solid-phase sandwich enzyme-linked immunosorbent assay (ELISA; Innotest hTau Ag®, Innogenetics, Zwijnaarde, Belgium). This ELISA measures total tau protein (normal tau and phosphorylated tau) by three monoclonal antibodies. The detection limit is 60 pg/mL. Values below the detection limit were set to zero for further statistical analyses. Intra- and inter-assay coefficients of variation were below 6%. NSE concentrations were measured with an immunoluminometric sandwich assay using directly coated magnetic microparticles (LIAISON, DiaSorin Diagnostika, Dietzenbach, Germany). LIAISON NSE® measurement is based on monoclonal antibodies that bind to the γ-subunit of the enzyme. The minimally measurable concentration is 0.04 μg/L. Intra- and inter-assay coefficients of variation were <3 and <6%, respectively. According to the manufacturer, the reported range in 95% of healthy subjects is <12.5 μg/L. A biological variation by gender and age was ruled out recently [20]. Previous studies that analysed various assays and a control group versus patients with acute ischemic stroke have reported significantly lower NSE concentrations in controls with a range from 6.3 (S.D. 1.6) μg/L up to 9.11 (S.D. 0.73) μg/L [2,10,11]. However, measurement of NSE serum concentrations were based on various techniques as in-house RIA and commercially available ELISA. None of those used a fully automated technique. Besides, the control groups in these studies were rather small and varied between 24 and 77 subjects. Due to these limitations, we abandoned an own control group and used the generally applied "conservative" cut-off value of 12.5 μg/L. This cut-off value represents the 95 percentile of healthy subjects investigated with the LIAISON. Laboratory investigators were blinded to clinical data.

All patients underwent a standardised admission and follow-up procedure. Extracranial and transcranial color-coded duplex sonography was performed in all patients on admission. In case of occlusion or severe stenosis of the internal carotid or middle cerebral artery, patients were re-examined 6 and 12 h (to 24 h) after stroke onset and before discharge. Middle cerebral artery main stem (proximal M1-segment) occlusion was diagnosed in the absence of a corresponding flow signal also after contrast enhancement. Diagnosis of distal middle cerebral artery main stem occlusion or multiple branch occlusion was based on an asymmetry index >21% [21]. Recanalisation of an occluded vessel segment was classified as absent, partial (visualization of a color-coded flow signal with reduced flow velocity and/or dampened pulsatility) or complete (normal/increased flow velocity and normal pulsatility) [22].

A CT scan was routinely performed before study inclusion to exclude patients with intracranial/intracerebral hemorrhage. All patients underwent CT or MRI control after 24 h and after 7–10 days. Infarction volume was calculated on all scans using the public domain United States National Institutes of Health (NIH) image program. Data on reliability and interrater agreements of measurements are given elsewhere [5].

Neurological deficits were quantified by the NIH stroke scale [23] on admission, Day 1, 4, 10–12, and at discharge and were performed by NIH-certified examiners. Functional disability was rated with the modified Rankin scale (mRS) [24] and with the Barthel index [25] on admission, Day 1, 4,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1017
000002

10–12, 90, and at discharge (median 12 days, range 2–35). For outcome analysis, the 90-day outcome was dichotomized into maximally mild disability with independence from help in the activities of daily life (mRS 0–2) and dependence or death (mRS 3–6).

Therapy was not randomised due to the observational character of the study. Selection of patients for intravenous thrombolysis and performance of thrombolysis was in accordance with approval criteria of the health authorities and started within 3 h. Conservative medical treatment included antiplatelet agents as primary drug therapy.

Statistical analyses were performed with the SPSS 10.0 program package (SPSS Inc.) and the MedCalc 5.0 statistical software (MedCalc Software). Differences between groups were analysed by nonparametric tests (χ^2-test or Fisher's exact test for smaller groups if appropriate and Mann–Whitney *U*-test). The threshold for significance was set at $P < 0.05$. In case of multiple comparisons, we applied an α-adjustment (Bonferroni correction) to compensate for type 1 errors.

3. Results

Sixty-six patients (39 men, 27 women) with a mean age of 61 (S.D. 14) years and acute stroke of the middle cerebral artery territory were included. Median NIH stroke scale score on admission was 13 (range 1–37). According to the TOAST criteria [26], stroke etiology was classified as cardioembolic (31 patients), large artery occlusion (14), small artery occlusion (5) and undetermined (16), the latter mostly due to concurrent pathologies. One half (33) of the patients were treated with intravenous rt-PA 139 min (mean, S.D. 25) after the onset of symptoms, and the other half (33) patients received antiplatelet agents as primary drug therapy. For further details see Table 1.

Table 1
Demographic, clinical and neuroradiological data of 66 patients with acute ischemic stroke of the middle cerebral artery

Patients, *n*	66
Age, mean (S.D.)	61 (14)
Male/female, *n* (%)	39 (59)/27 (41)
Time from onset to admission median (range)	180 (60–300)
NIH-SS score, median (range)	
On admission	14 (1–37)
Day 1	11 (0–34)
Day 4	6 (0–32)
At discharge	5 (0–41)
Barthel index, median (range)	
At discharge	65 (0–100)
Modified Rankin scale, median (range)	
After 90 days	3 (0–6)
Favourable (mRS 0–2), *n* (%)	36 (55)
Poor (mRS 0–2), *n* (%)	30 (45)
Thrombolysis, *n* (%)	33 (50)
Conservative treatment, *n* (%)	33 (50)
Infarct volume (mL), median (range)	52 (0–498)
Etiology (TOAST criteria), *n* (%)	
Cardioembolism	31 (47)
Large-artery occlusion	14 (21)
Small-artery occlusion	5 (8)
Undetermined	16 (24)

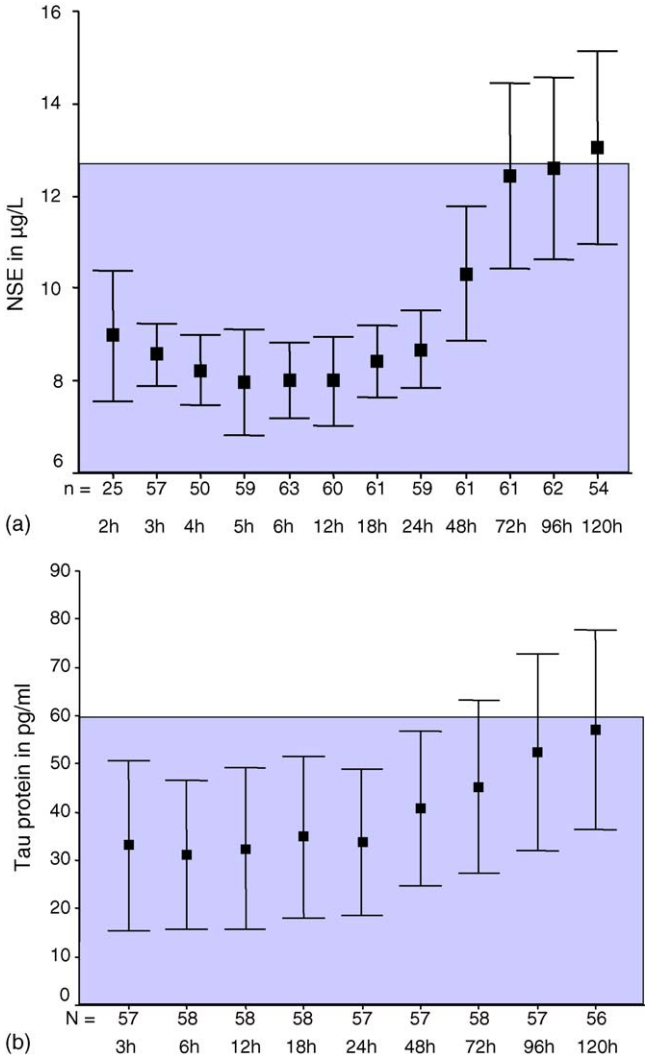

Fig. 1. (a) Release of NSE in acute stroke. Data shown as means ± 95% CI. Shaded areas indicate the respective reference range (12.5 µg/L) and (b) release of tau protein in acute stroke. Data shown as means ± 95% CI. Shaded areas indicate the respective reference range (60 pg/mL).

Fig. 1 shows the release patterns of NSE and tau protein. After a first rise 2–3 h after onset of first stroke symptoms, NSE decreased until 12 h, followed by a secondary increase until last measurement on Day 5 (Wilcoxon test: $z = -3764$, $P < 0.001$). Within the first 24 h only 13% of NSE concentrations but within 5 days 43% were found above the cut-off value of 12.5 µg/L. Tau protein concentrations showed a continuous increase from admission to last measurement 120 h after stroke (Wilcoxon test: $z = -3782$, $P < 0.001$). In 27% of our patients tau protein values were elevated (above 60 pg/mL) within the first day and in 41% until Day 5. None

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1017
000003

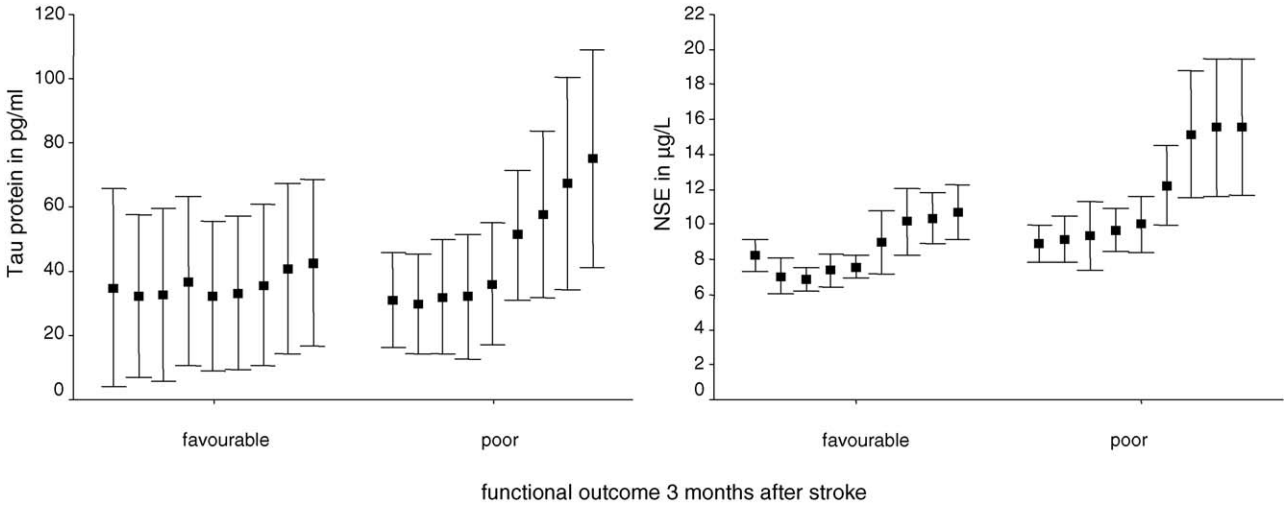

Fig. 2. Mean values ±95% CI of NSE and tau protein in patients with favourable functional outcome (mRS 0–2, $n = 36$) and poor functional outcome with dependency in the activities of daily living (mRS 3–6, $n = 30$). Columns within each group represent measurement time points from admission to 120 h. after stroke onset.

of our patients exhibited signs of renal dysfunction/renal failure or muscle trauma/disease (creatine kinase was normal). Significant intercorrelations between NSE and tau protein were not found (Fig. 2).

Highest serum concentrations of NSE and tau protein were recorded in patients with large infarctions. Volume of finally infarcted brain as visible on cranial CT or MRI and serum concentrations were significantly correlated from 12 h (NSE) and from 72 h (tau protein) after stroke onset onward. Highest correlations were found between release over time as assessed as area under curve (AUC) and infarction volume (NSE_{AUC}: $r = 0.762$, $P < 0.0001$; tau_{AUC}: $r = 0.320$, $P = 0.01$). Serum concentrations of NSE and tau protein did not differ significantly with respect to stroke etiology as assessed with the TOAST-criteria [26]. No significant dependence of serum values was found in relation to lesion location.

NSE serum concentrations from 18 h onward were highly correlated with the severity of the corresponding neurological deficit as quantified by the NIH stroke scale from Days 1, 4, 12 and at discharge. Highest correlation was found for NSE and corresponding NIH-SS on Day 4 ($NSE_{Day4} - NIH_{Day4}$ $r = 0.630$, $P = 0.0001$). Also, tau protein values from 48 h onward correlated with the severity of clinical deficit. The highest correlation was found for tau protein peak values and NIH on Day 4 ($tau_{peak} - NIH_{Day4}$ $r = 0.442$, $P = 0.001$) but a high correlation was also found for tau protein and corresponding NIH-SS on Day 4 ($tau_{Day4} - NIH_{Day4}$ $r = 0.438$, $P = 0.001$).

The neurovascular status on admission revealed occlusion of the middle cerebral artery main stem (M1-segment) in 35 patients (6 with additional distal internal carotid, 7 with additional carotid-T occlusion), multiple branch occlusions in 9 patients and normal findings in 22 patients, compatible with an occlusion of a single branch or smaller artery. A pathological neurovascular status on admission resulted in a significantly higher release of NSE from 48 h onward ($P = 0.001$), but no association to early or late recanalisation after intravenous thrombolysis was found. Tau protein concentrations were also elevated in case of basal vessel occlusion. However, this difference did not reach statistical significance. Neither NSE nor tau protein values differed between patients who underwent intravenous thrombolysis or conservative medical treatment. Patients with complete recanalisation after thrombolysis exhibited lower serum levels, but this did not reach statistical significance.

At discharge, functional outcome evaluated by the Barthel index (median 65, range 0–100) was significantly correlated with NSE concentrations from 12 h until last measurement 120 h after stroke onset with highest correlations at 120 h ($r = 0.559$, $P < 0.001$). Also, late tau protein values from 48 to 120 h onward were significantly associated with the Barthel index at discharge with maximal correlation on Day 5 ($r = 0.407$, $P = 0.002$).

Functional outcome at 3 months as assessed by the mRS was favorable in 36 patients (mRS 0 in 9; mRS 1 in 10; mRS 2 in 13) and poor in 30 (mRS 3 in 12; mRS 4 in 14; mRS 5 in 3; mRS 6 in 5). One patient died in hospital 13 days after stroke due to septicemia and 4 patients due to pneumonia in the rehabilitation unit or nursing home. Serum concentrations of NSE from 12 h onward and tau protein values from 48 h onward were associated with outcome at 3 months with a maximal association obtained for NSE at 96 h ($NSE_{96\,h}$: $r = 0.443$, $P < 0.001$) and tau protein at 120 h ($tau_{120\,h}$: $r = 0.422$, $P = 0.001$) indicating that patients with an adverse functional outcome had a higher increase of NSE and tau protein.

At these time points, NSE values above 14.85 µg/L (cut-off value obtained from receiver operator curves: sensitivity 89%, specificity 42%, positive likelihood ratio 1.55, negative likelihood ratio 0.25) and tau protein concentrations above

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1017
000004

77.85 pg/mL (sensitivity 90%, specificity 40%, positive likelihood ratio 1.51, negative likelihood ratio 0.24) were strong indicators of a poor functional status 3 months after stroke.

4. Discussion

NSE serum concentrations showed a first rise 2–3 h after onset of first stroke symptoms. Afterwards, NSE decreased, followed by a secondary increase until last measurement on Day 5. This release pattern is in accordance with previous studies of serum NSE in acute stroke in humans [5,12,27] and plasma and CSF investigations in rat stroke models [9,28,29] indicating no discrepancy between the different NSE assays. However, in contrast to Hill et al. [30] who reported elevated NSE levels in 89% of their patients on admission with an unspecified ELISA, only 13% of NSE concentrations within the first 24 h and in 43% within the first 5 days were found above the cut-off value of 12.5 μg/L in our study.

Tau protein concentrations in serum showed a continuous increase from admission to last measurement 120 h after stroke and were elevated in 27% of our patients within the first day and in 41% until Day 5. In accordance to Bitsch et al. [19], in patients with an early rise of serum concentrations, values remained elevated. On the other hand, 10 patients showed a late increase after Day 1 until Day 5. These findings support data on tau protein release into CSF in acute stroke by Hesse et al. [18].

Correlations between the release of NSE and tau protein and infarction volume indicate that serum concentrations reflect the amount of ischemic brain damage, confirming that both markers derive out of neuronal brain damage. However, missing intercorrelations between NSE and tau protein indicate different release mechanisms. In analogy to previous studies on humans and animals, the early rise of NSE and tau protein in blood within a few hours after ischemic stroke onset may reflect a rapid release out of initially damaged neuronal tissue, and can be explained by the known early elevated CSF concentrations [9,28], which result in a locally increased brain–blood gradient and passage through the blood brain barrier into systemic circulation by molecular diffusion and the known CSF/serum ratios of NSE (1:1) and tau protein (10:1) [31]. After exclusion of renal dysfunction/renal failure or muscle trauma/disease as a possible explanation in our patients, the secondary increase indicates further release of NSE and tau protein, which probably reflects secondary mechanism of brain damage, ongoing neuronal cell death [5,9,12,29] or persistent disturbance of the blood–brain barrier [32].

Furthermore, our data show a strong correlation between serum concentrations of NSE from 12 h onward and the corresponding neurological status as assessed by the NIH stroke scale score, supporting previous studies [5,6,11,30] on the correspondence between neurological deficits and release of NSE in serum. Only one single study of 24 stroke patients failed to show a significant correlation of NSE (RIA) and the Scandinavian stroke scale score, even though highest NSE concentrations were found in severe stroke with fatal outcome [2]. However, in contrast to the previous study on tau protein [19], our study also indicates a significant association between tau protein release and the early clinical course. A deteriorating neurological status was associated with higher serum levels and improvement of the neurological deficit with lower values. These data indicate that serum NSE and tau protein could be clinically useful as a monitoring parameter in addition to clinical and neuroradiological information in acute ischemic stroke.

A pathological neurovascular status on admission resulted in a significantly higher release of NSE from 48 h onward ($P = 0.001$). Also, late tau protein concentrations were elevated in case of basal vessel occlusion (n.s.), but no association to early or late recanalisation after intravenous thrombolysis was found. Even though patients with complete recanalisation after thrombolysis showed lower serum levels, this did not reach statistical significance. The hypothesized rise of tau protein as a model of reperfusion [19] could not be confirmed. In contrast to protein S100B [6,7], the association between NSE and tau protein and the neurovascular status could be a consequence of larger infarction due to basal vessel occlusion. However, the correlation of serum concentrations from 48 h onward and the neurovascular status on admission is far too late from decision-making in acute stroke treatment.

Correlations between NSE and tau protein serum concentrations and functional disability at discharge and after 3 months may contain greater clinical potential. Whereas a correlation between the area under curve of tau protein and the Barthel index and the mRS after 3 months has been described before by Bitsch et al. [19], our study also revealed that tau protein values from 48 h onward were associated with the outcome at 3 months with a maximum of correlation obtained at 120 h and a poor functional outcome at tau protein concentrations above 77.85 pg/mL. Furthermore, correlations between NSE serum concentrations and functional disability have been described for the short-term outcome using the Barthel index at discharge in ischemic stroke [5] and can be confirmed by our results. Our study also could demonstrate a significant association between NSE concentrations and functional disability 90 days after stroke with a maximum of correlation at 96 h and a poor functional outcome at NSE concentrations above 14.85 μg/L.

Even though, these results are promising, some restrictions have to be considered. Our data result from a well-defined group of patients with a hyperacute ischemic stroke in the middle cerebral artery territory and a thrombolysis rate of 50% and therefore do not fully reflect clinical reality. Thus, generalization of our results to the general stroke population is not justified and should be reconfirmed in a larger stroke population. Furthermore, NSE is vulnerable to hemolysis and therefore the interpretation of hemolytic samples is hampered/not possible. Also, tau protein is measurable only in a proportion of the samples but this is a technical problem,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1017
000005

which could be solved through more sensitive analyses in future.

In summary, release kinetics of NSE and tau protein in ischemic stroke are associated with patient's clinical deficits and infarct volume, and may be used as an additional predictor of the early course and the functional outcome of stroke patients.

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Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1017
000006