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The role of brain territorial involvement and infection/inflammation in the long-term outcome of neonates with arterial ischemic stroke: A population-based cohort study

Eszter Vojcek [a,b,*], Agnes Jermendy [a], Anna M. Laszlo [c], Rozsa Graf [d], Gabor Rudas [e], Marianne Berenyi [f], Istvan Seri [a,g]

[a] First Department of Pediatrics, Semmelweis University, Budapest, Hungary
[b] Department of Pediatrics, Saint John Hospital and North-Buda Unified Hospitals, Budapest, Hungary
[c] Institute of Mathematics and Base Sciences, Szent István University, Budapest, Hungary
[d] Department of Rehabilitation, Szent János Hospital and North Buda United Hospitals, Budapest, Hungary
[e] Department of Neuroradiology, Medical Imaging Centre, Semmelweis University, Budapest, Hungary
[f] Department of Developmental Neurology, Saint Margaret Hospital, Budapest, Hungary
[g] Department of Pediatrics, Children's Hospital Los Angeles, University of Southern California, Los Angeles, United States

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ABSTRACT

Background: Neonatal arterial ischemic stroke (NAIS) carries the risk of significant long-term neurodevelopmental burden on survivors.
Aims: To assess the long-term neurodevelopmental outcome of term neonates diagnosed with NAIS and investigate the associations among brain territorial involvement on MRI, clinical risk factors and neurodevelopmental outcomes.
Study design: Population-based cohort study.
Subjects: Seventy-nine term neonates with NAIS confirmed by MRI born between 2007 and 2017.
Outcome measures: Long-term neurodevelopmental outcome assessed using the Bayley Scales of Infant Development-II, the Brunet-Lézine test and the Binet Intelligence scales-V.
Results: Follow-up was available in 70 (89%) of the subjects enrolled, at a median age of 60 months [IQR: 35–84]. Normal neurodevelopmental outcome was found in 43% of the patients. In a multivariable model, infants with main MCA stroke had an increased risk for overall adverse outcome (OR: 9.1, 95% CI: 1.7–48.0) and a particularly high risk for cerebral palsy (OR: 55.9, 95% CI: 7.8–399.2). The involvement of the corticospinal tract without extensive stroke also increased the risk for cerebral palsy/fine motor impairment (OR: 13.5, 95% CI: 2.4–76.3). Multiple strokes were associated with epilepsy (OR: 9.5, 95% CI: 1.0–88.9) and behavioral problems (OR: 4.4, 95% CI: 1.1–17.5) and inflammation/infection was associated with cerebral palsy (OR: 9.8, 95% CI: 1.4–66.9), cognitive impairment (OR: 9.2, 95% CI: 1.8–47.8) and epilepsy (OR: 10.3, 95% CI: 1.6–67.9).
Conclusions: Main MCA stroke, involvement of the corticospinal tract, multiple strokes and inflammation/infection were independent predictors of adverse outcome, suggesting that the interplay of stroke territorial involvement and clinical risk factors influence the outcome of NAIS.

1. Introduction

The pregnant woman and her fetus have risk factors that shift the balance of the fetal coagulation homeostasis toward a prothrombotic state [1]. The resulting unique fetal-neonatal coagulation status has been postulated to contribute to the development of perinatal stroke

Abbreviations: NAIS, neonatal arterial ischemic stroke; CP, cerebral palsy; MRI, magnetic resonance imaging; MCA, middle cerebral artery; ACA, anterior cerebral artery; PCA, posterior cerebral artery; CST, corticospinal tract; PLIC, posterior limb of the internal capsule; BSID-II, Bayley Scales of Infant Development, Second Edition; SD, standard deviation; IQR, interquartile range; OR, odds ratio; CI, confidence interval.

* Corresponding author at: First Department of Pediatrics, Semmelweis University, Bókay J. u. 53-54, Budapest 1083, Hungary.
E-mail address: vojcek.eszter@med.semmelweis-univ.hu (E. Vojcek).

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presenting as perinatal ischemic stroke or perinatal hemorrhagic stroke. Perinatal ischemic stroke has been defined as a focal disruption of cerebral blood flow that occurs between 20 weeks of gestation and 28 days after delivery and carries the risk of chronic neurological sequelae [1]. Based on the type of the vessel affected, the two major forms of perinatal ischemic stroke are arterial ischemic stroke and cerebral sinovenous thrombosis. Based on the time of presentation, arterial ischemic stroke with acute presentation during the first 28 postnatal days has been classified as neonatal arterial ischemic stroke (NAIS) while stroke with delayed presentation is identified as presumed perinatal arterial ischemic stroke [1–3]. The latter is usually diagnosed by imaging studies performed after the first postnatal month with findings suggestive of a stroke of perinatal origin [1].

In neonatal arterial ischemic stroke (NAIS), cerebral vessels are most commonly occluded by thrombosis or embolism [4]. The estimated incidence of NAIS is one in 2300 to 5000 live birth [5–7]. Therefore, stroke is 17 times more common in the perinatal period than any time later in childhood [8].

Neonatal arterial ischemic stroke is an important cause of long-lasting neurodevelopmental sequelae including hemiparetic cerebral palsy (CP) [9,10], cognitive [11] and language impairment [12], epilepsy [13], and/or behavioral disorders often manifesting only at school age [14].

Long-term follow-up studies of NAIS patients are limited and population-based, longitudinal studies are lacking. Therefore, we designed a population-based cohort study, involving all patients born over an 11-year period in Central-Hungary. Our aim was to investigate the long-term neurodevelopmental outcomes of all patients diagnosed with NAIS and to describe the possible associations among clinical presentation, potential clinical risk factors, magnetic resonance imaging (MRI) findings and long-term neurodevelopmental outcome.

2. Patients and methods

2.1. Population

This is a population-based cohort study designed to capture all term neonates with NAIS born between January 1, 2007 and December 31, 2017 and cared for in the Central-Hungarian region, including the capital, Budapest. MRI scans were performed at a single center, in the Department of Neuroradiology, Medical Imaging Center, Semmelweis University, Budapest, Hungary. We reviewed the imaging studies of 1400 term neonates (≥37 weeks) who had a head MRI performed prior to 28 days of life and identified 102 subjects with evidence of a focal cerebral arterial ischemic event. Patient selection is shown in Fig. 1. Inclusion criteria were: (1) term neonates up to 28 days of age (2) and a brain MRI confirming the diagnosis of NAIS without any other concurrent abnormality. We included neonates with congenital heart disease with the exception of those with single ventricle physiology, since congenital heart disease is a major risk factor for stroke [5]. We excluded neonates with kernicterus, encephalitis, border-zone injury or periventricular leukomalacia on MRI, birth asphyxia, tumor, non-accidental brain injury, mitochondrial disorders and congenital syndromes with known adverse outcome [4]. We obtained Institutional Review Board

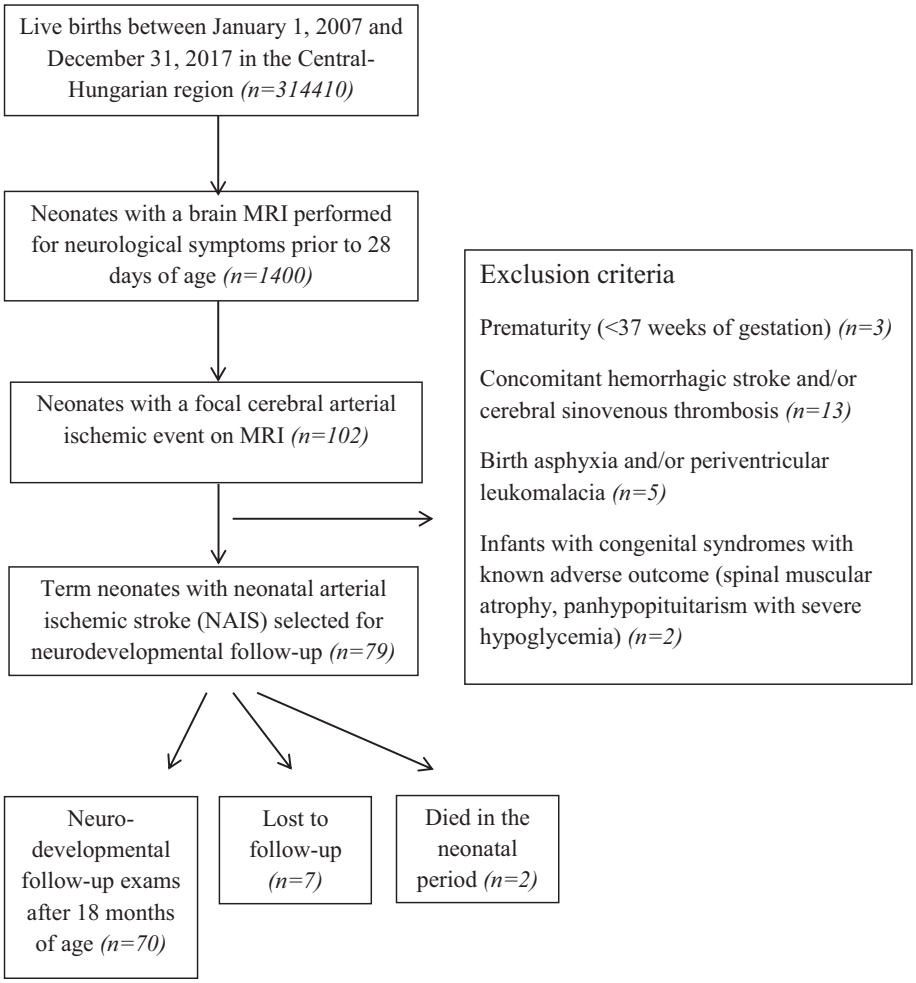

Fig. 1. Process of patient selection, inclusion and exclusion.

approval from the Hungarian Medical Research Council (19934–4/2018/EKU). Informed verbal parental consent was obtained to recruit patients into the study and to perform neurodevelopmental assessments.

2.2. MRI

Brain imaging was carried out on 3 T Philips Achieva and 3 T Philips Ingenia MR scanners (Philips Medical Systems, Best, The Netherlands) at the Department of Neuroradiology, Medical Imaging Center, Semmelweis University, Budapest, Hungary. The scanning protocol included diffusion-weighted imaging, apparent diffusion coefficient map, conventional T1- and T2-weighted imaging, between 2007 and 2009 T2*- and between 2009 and 2017 susceptibility-weighted imaging. In selected cases, MR angiography and/or MR spectroscopy were added, as appropriate. Radiologists trained and experienced in neonatal brain MRI evaluated the MRI scans. Classification was based on the shape, extent and localization of the affected brain compartment supplied by the given arteries [4,15]. Each stroke was assigned to one of the most predominant arterial territory (for further details see the Supplemental Methodology Statement 1, Figure Supplementary Fig. 1 [16]). The involvement of more than one branch of the middle cerebral artery (MCA) was designated as multiple lesions. Previous reports have described an association between involvement of the corticospinal tract (CST) and adverse neurodevelopmental outcome including CP [17–19]. Therefore, we also investigated the involvement of the CST seen as "pre-Wallerian degeneration" on diffusion-weighted imaging at the level of the posterior limb of the internal capsule (PLIC) and cerebral peduncles. Strokes of <3 mm in size at the largest diameter were not included in this study.

2.3. Clinical data

Clinical information was collected from chart reviews. Gestational age, birthweight, sex, modes of delivery, Apgar scores, seizures, hypoglycemia (defined as blood glucose level < 2.6 mmol/L), need for resuscitation and/or mechanical ventilation and/or cardiovascular support, abnormal muscle tone and level of consciousness (irritability/lethargy) along with other neonatal neurological symptoms were recorded. Specific or non-specific inflammation was defined as positive blood and/or cerebrospinal fluid culture or clinical signs of inflammation along with a CRP of >10 mg/L, respectively. Established risk factors for NAIS during pregnancy, such as gestational diabetes, preeclampsia and placental abruption, were collected retrospectively. We performed electroencephalogram (EEG) studies on 64 patients based on the clinical findings. The EEG signals were classified as seizure, abnormal background activity (including low voltage, flat trace, burst suppression, trace alternant, asynchrony or asymmetry) or normal EEG [20]. Echocardiography was used as indicated to confirm/rule out congenital heart disease.

2.4. Neurodevelopmental outcome

In all patients, neurodevelopmental outcome was assessed during follow-up visits between the age of 1.5 to 10 years. For the purposes of this study, we also prospectively performed a systematic neurodevelopmental follow-up assessment of children that were diagnosed with NAIS and were available for follow-up in 2018. Normal outcome was defined as symptom free survival. Adverse outcome was noted if any one or more of the following sequelae occurred: CP, fine motor impairment, cognitive impairment, behavioral problems, epilepsy, language delay, visual field defect or hearing loss. The developmental tests used during the follow-up visits and the diagnostic criteria for CP and epileptic syndromes are described in the Supplemental Methodology Statement 2 in detail [21–26].

2.5. Statistical analysis

Descriptive statistics were expressed as frequencies and percentages, mean and standard deviation (SD) or median and interquartile range [IQR], as appropriate. Stroke subtypes and EEG findings were compared with clinical symptoms and outcome parameters by using Fisher's exact tests. Univariate logistic regression model was used to describe relationships between clinical predictors of interest and neurodevelopmental outcome. Multivariable logistic regression model was applied to ascertain the effect of significant predictors including infection/inflammation, main MCA stroke and multiple strokes based on the notion that a patient would likely have an adverse outcome, while controlling for other clinically relevant factors based on the results of the univariate analysis and a priori knowledge. Finally, univariate and multivariable regression analyses were repeated after excluding patients with main branch MCA infarction to better determine the risk for adverse outcome of neonates with CST injury without extensive stroke. Odds ratios (OR) with 95% confidence intervals (CI) were calculated for each clinical variable. Two-sided statistical tests with p values of <0.05 were considered significant. Statistical analyses were performed using IBM SPSS Statistics (Version 25, IBM Corp. Armonk, NY, USA).

3. Results

3.1. Patient characteristics and general findings

A total of 79 neonates diagnosed with acute NAIS were identified in Central Hungary over the 11-year study period, yielding a disease incidence of 1 per 3800 live births. Clinical characteristics, maternal and neonatal risk factors and presenting symptoms of the study population are summarized in Table 1.

The most common presenting symptom of NAIS was seizure activity, occurring in 75% of patients, at a median age of 2 days [IQR 1–3]. As expected, seizures were of focal onset in the majority of patients. The rate of hypoglycemia was also high, detected in 25% of the patients.

Table 1
Clinical characteristics, maternal and neonatal risk factors and presenting symptoms of neonatal arterial ischemic stroke in the study population. Frequencies and percentages, mean and standard deviation (SD) or median and interquartile range [IQR] were calculated, as appropriate.

Clinical characteristics, risk factors and presenting symptoms	Patients with NAIS *n* = 79
Gestational age [weeks], *mean (SD)*	38.8 (1.4)
Birthweight [g], *mean (SD)*	3312 (592)
Male, *n (%)*	50 (58)
Apgar score 1 min, *mean (SD)*	7 (3)
Apgar score 5 min, *mean (SD)*	9 (2)
Gestational diabetes, *n (%)*	8 (11)
Preeclampsia, *n (%)*	13 (16)
Placental abruption, *n (%)*	17 (22)
C-section, *n (%)*	42 (53)
5-min Apgar score < 7, *n(%)*	9 (11)
Congenital heart disease, *n (%)*	12 (15)
Cranial arteriopathy, *n (%)*	9 (11)
Infection/Inflammation, *n (%)*	15 (19)
Blood stream infection	4
Meningitis	2
Suspected infection with CRP > 10 mg/L	9
CRP in patients with infection/ inflammation, *median [IQR]*	36 [13.5–85.5]
Clinical seizure, *n (%)*	59 (75)
Postnatal day at first seizure, *median [IQR]*	2 [1–3]
Hypoglycemia[a], *n (%)*	20 (25)
Irritability/lethargy, *n (%)*	10 (13)
Muscle tone abnormalities, *n (%)*	13 (16)
Respiratory distress, *n (%)*	29 (37)
Need for complex resuscitation and/or mechanical ventilation and/or cardiovascular support, *n (%)*	7 (9)

[a] Hypoglycemia was defined as blood glucose level < 2.6 mmol/L.

3.2. Affected brain regions

Diagnostic brain MRI was performed at a median age of 5 [IQR 3–7] days. The most commonly affected brain regions were the areas supplied by the main ($n = 16$, 20%), the anterior ($n = 17$, 22%) and the posterior branches of the MCA ($n = 15$, 19%). Stroke was more often left sided ($n = 38$, 48%) and, similarly to findings in the literature multiple strokes ($n = 33$, 42%) were common. Involvement of the CST was described in 28 neonates (35%). Detailed frequencies of stroke subtypes are shown in Table 2.

Analysis of associations between clinical characteristics and stroke subtypes revealed that hypoglycemia was more often found in PCA strokes compared with the other subtypes (56% vs. 21%, $p = 0.04$). Muscle tone abnormalities were noted more frequently in strokes of the perforator arteries (43% vs. 8%, $p = 0.005$). Neonates with infection/inflammation did not have significantly higher rate of cerebral arteriopathy compared to neonates without evidence of infection/inflammation (20% vs. 9%, $p = 0.5$). In addition, patients with infection/inflammation did also not have more areas of infarct or higher rates of main branch MCA stroke than the other subgroups (54% vs. 44%, p = 0.5 and 31% vs. 19%, p = 0.5, respectively).

3.3. Neurodevelopmental outcome

Long-term neurodevelopmental outcome data were available in 70 (89%) of the 79 patients with the last follow-up visit occurring at a median age of 60 [IQR 35–84] months. Of the 9 patients not included in the analysis, two died from complications of stroke and congenital heart disease in the neonatal period, three had follow-up examinations between 12 and 18 months of age with normal neurodevelopment but were lost to follow-up thereafter; and four were lost to follow-up after the neonatal period. All infants lost to follow-up had small, 5–7 mm stroke territorial involvement on MRI not involving the basal ganglia and thalami.

Thirty of the 70 patients (43%) with longitudinal follow-up had normal neurodevelopmental outcome. Among the subjects with adverse neurodevelopmental outcomes, CP was the most frequent finding, documented in 20 patients (29% of all patients with follow-up). Thus, among the 40 subjects with adverse neurodevelopmental outcomes, 50% were diagnosed with CP. Of them, 17 children (85% of patients with CP) had unilateral hemiparesis and the remaining three children (15% of patients with CP) presented with tetraparesis. In addition to CP, fine motor impairment (13%), as well as cognitive deficit (17%), behavioral problems (21%, two-thirds of whom had attention deficit hyperactivity disorder) and speech delay (23%) were common. Active epilepsy was documented at the time of assessment in 7 patients (10%). Hearing loss and visual field defects were rare (4% and 3%, respectively).

To further assess the co-occurrence of the adverse outcome variables, we created three main categories and plotted patients on a Venn diagram: (1) CP and/or fine motor impairment, (2) cognitive impairment and/or behavioral problems, (3) language delay and/or visual field defects and/or hearing loss and we also included patients with epilepsy (Fig. 2). Even though more than one type of adverse neurodevelopmental outcome was recorded in 18 subjects (26%), the co-occurrence of adverse outcome measures in the three major domains (motor skills, cognitive field and sensory/language field) was relatively low ($n = 8$, 11%). The rate of severe CP was low ($n = 3$, 4%) and moderate-to-severe global intellectual deficit was also relatively rare ($n = 7$, 10%).

Interestingly, we detected an association between cognitive impairment *and* epilepsy ($p = 0.04$) and language delay ($p = 0.004$). Moreover, we also found associations between behavioral problems *and* cognitive impairment ($p = 0.016$) and language delay ($p = 0.003$). Finally, CP was

Table 2
MRI findings of territorial involvement and specific arteries affected by NAIS and adverse outcome domains per stroke territory subtypes. Frequencies of specific neurological outcome domains per stroke territory subtypes were calculated in infants with long-term follow-up.

NAIS types and outcomes	Total n = 79, *(n %)*	Follow-up n = 70, *(n%)*	CP *n* = 20, *(n%)*	Fine motor impairment *n* = 9, *(n%)*	Cognitive impairment *n* = 12, *(n%)*	Behavioral problems n = 15, *(n%)*	Language/visual/ hearing problems *n* = 18, *(n%)*	Epilepsy n = 9, *(n%)*	Overall adverse outcome *n* = 40, *(n%)*
Arterial involvement									
Main MCA	16 (20)	15 (21)	13 (87)	3 (20)	6 (40)	7 (47)	9 (60)	5 (33)	14 (93)
Anterior MCA	17 (22)	16 (23)	3 (19)	1 (6)	2 (13)	0 (0)	3 (19)	1 (6)	8 (50)
Middle MCA	11 (14)	11 (16)	1 (9)	0 (0)	1 (9)	1 (9)	2 (18)	0 (0)	4 (36)
Posterior MCA	15 (19)	12 (17)	1 (8)	2 (17)	0 (0)	6 (50)	2 (17)	1 (13)	8 (67)
Perforator stroke	9 (11)	7 (10)	1 (14)	0 (0)	1 (14)	0 (0)	1 (14)	1 (8)	3 (43)
PCA/ACA	12 (15)	9 (13)	1 (11)	3 (33)	2 (22)	1 (11)	1 (11)	1 (11)	4 (44)
Multiple strokes	33 (42)	32 (46)	13 (41)	5 (16)	7 (22)	11 (34)	11 (34)	7 (22)	23 (72)
Territorial involvement									
PLIC +/− cerebral peduncles	28 (35)	27 (39)	17 (63)	6 (22)	8 (30)	9 (33)	13 (48)	6 (22)	24 (89)
PLIC +/− cerebral peduncles without main branch MCA[a]	12 (15)	12 (17)	4 (33)	3 (25)	2 (17)	2 (17)	4 (33)	1 (8)	10 (83)
Side of stroke lesion									
Right stroke	23 (29)	20 (29)	6 (30)	3 (15)	6 (30)	6 (30)	6 (30)	4 (20)	9 (45)
Left stroke	38 (48)	35 (50)	9 (26)	5 (14)	2 (6)	3 (9)	5 (14)	3 (9)	20 (57)
Bilateral strokes	18 (23)	15 (21)	5 (33)	1 (7)	4 (27)	6 (40)	7 (47)	2 (13)	11 (73)

Percentages are shown in brackets.
Abbreviations: MCA: middle cerebral artery, PCA: posterior cerebral artery, ACA: anterior cerebral artery, PLIC: posterior limb of internal capsule, CP: cerebral palsy.
[a] Frequencies calculated in patients after excluding those with main branch MCA stroke.

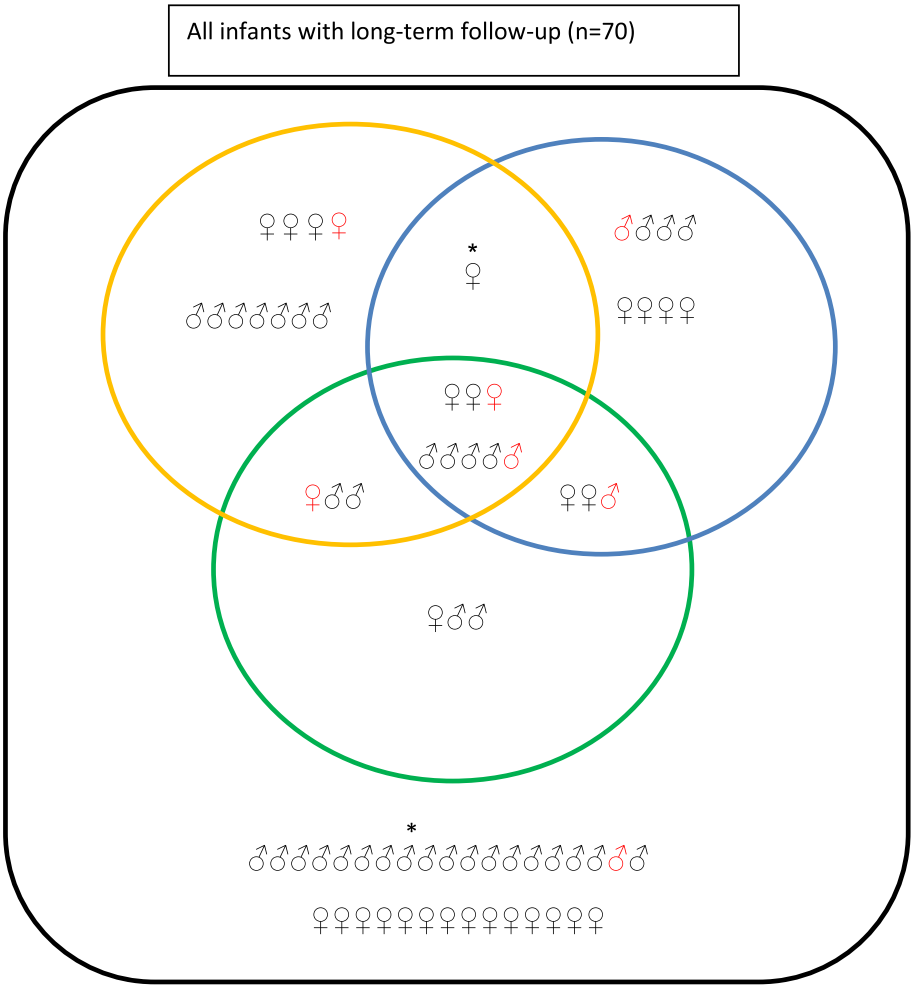

Fig. 2. Venn diagram of all infants with NAIS and long-term follow-up results (n = 70). Neurodevelopmental outcome was assessed after 18 months of age in all patients. Female and male symbols represent single patients. Patients are categorized as follows: *yellow circle*: cerebral palsy and/or fine motor impairment; *blue circle*: cognitive impairment and/or behavioral problems; *green circle*: language delay and/or visual field defects and/or hearing loss. Symbols in red indicate patients with active epilepsy, symbols with an asterix indicate patients who are seizure-free for more than 1 year without treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Results of multivariable logistic regression analysis represented by adjusted odds ratios and 95% confidence intervals predicting the likelihood of adverse outcomes in patients with NAIS and main branch MCA stroke and/or inflammation/infection (**A**) and predicting the likelihood of adverse outcomes in patients with NAIS and multiple strokes (**B**).

A

	Cerebral palsy	Cognitive impairment	Behavioral problems	Epilepsy	Visual/hearing/language problems	Adverse outcome
Infection/Inflammation, aOR [95% CI]	9.8 [1.4–66.9]	9.2 [1.8–47.8]	NS	10.3 [1.6–67.9]	NS	NS
Main MCA, aOR [95% CI]	55.9 [7.8–399.2]	6.4 [1.2–33.3]	8.8 [1.9–41.4]	17.3 [2.0–153.5]	9.7 [2.3–41.4]	9.1 [1.7–48.0]
Hypoglycemia, aOR [95% CI]	NS	NS	NS	NS	NS	NS
Seizure, aOR [95% CI]	NS	NS	NS	NS	NS	NS
Need for resuscitation, aOR [95% CI]	NS	NS	NS	NS	NS	NS

B

	Cerebral palsy	Behavioral problems	Epilepsy	Adverse outcome
Infection/inflammation, aOR [95% CI]	4.9 [1.1–21.6]	NS	10.6 [1.7–67.9]	NS
Multiple strokes, aOR [95% CI]	NS	4.4 [1.1–17.5]	9.5 [1.0–88.9]	3.2 [1.1–9.2]
Hypoglycemia, aOR [95% CI]	NS	NS	NS	NS
Seizure, aOR [95% CI]	NS	NS	NS	NS
Need for resuscitation, aOR [95% CI]	NS	NS	NS	NS

Abbreviations: CI: confidence interval, aOR: adjusted odds ratio, NS: non-significant.

associated with epilepsy, language delay and cognitive impairment (p = 0.01, $p < 0.001$ and $p = 0.002$, respectively).

3.4. Risk factors associated with adverse neurodevelopmental outcome

Univariate analysis of stroke territory subtypes and their relation to neurodevelopmental outcome domains revealed several associations between MRI parameters and neurodevelopmental outcome domains (Supplemental Table 1A). Main MCA stroke, multiple strokes and the involvement of the CST were associated with increased odds for overall adverse outcomes. Indeed, 93% of patients with main branch MCA stroke had neurological impairment, compared to 29% to 67% of other NAIS subtypes and the rate of CP was especially high (87%). After excluding neonates with main branch MCA stroke, patients with CST involvement still had a high rate of adverse neurodevelopmental outcome (83%) compared to those without CST involvement (37%). Among the clinical risk factors, evidence for infection/inflammation conferred significantly higher odds for adverse outcome in several developmental domains (Supplemental Table 1B).

Multivariable logistic regression analysis revealed that the presence of main MCA stroke and signs of infection/inflammation were significant independent predictors of impairment in several neurodevelopmental domains (Table 3A). Infants with main MCA stroke had 9.1 times higher odds (95% CI: 1.7–48.0) for overall adverse outcome, and main branch MCA infarction was associated with CP, cognitive and behavioral problems, epilepsy and visual/hearing/language problems. Interestingly, infection/inflammation was associated with adverse outcome only in certain neurodevelopmental domains, including a higher likelihood for CP, cognitive deficit and epilepsy. As main MCA stroke and multiple strokes showed collinearity ($R = 0.6$, $p < 0.05$), we repeated the multivariable analysis with the same independent variables except for replacing main branch MCA stroke with multiple strokes and found multiple strokes to be significantly associated with epilepsy (OR: 9.5; 95% CI: 1.0–88.9) and behavioral problems (OR: 4.4; 95% CI: 1.1–17.5) (Table 3B). Finally, after excluding patients with main branch MCA stroke, the involvement of PLIC with or without cerebral peduncles was associated with an increased risk for overall adverse outcome (OR: 6.5; 95% CI: 1.1–36.6) and CP/fine motor impairment (OR: 13.5; 95% CI: 2.4–76.3) (Table 4).

Of the patients who had EEG studies done on admission ($n = 64$, 81%) and were available for long-term follow-up ($n = 58$, 73%), normal EEG was recorded in 26 of the 58 patients (45%), seizure activity was detected in 19 (33%) infants and abnormal background activity was recognized in 17 (29%) subjects. Interestingly, neonates with seizure activity on EEG developed later epilepsy more frequently compared to those without seizure activity on EEG (26.3% vs. 8.3%, $p = 0.05$). Additionally, patients with abnormal background activity on EEG had adverse neurodevelopmental outcome more often compared to those without abnormal background activity on EEG (70.6% vs. 48.8%, p = 0.05).

Table 4
Results of multivariable logistic regression analysis represented by adjusted odds ratios and 95% confidence intervals predicting the likelihood of overall adverse outcome and cerebral palsy/fine motor involvement in patients after excluding those with main branch MCA stroke.

	Cerebral palsy	Cerebral palsy/fine motor involvement	Adverse outcome
PLIC +/− cerebral peduncles	NS	13.5 [2.4–76.3]	6.5 [1.1–36.6]
Infection/inflammation, aOR [95% CI]	8.5 [1.1–67.1]	NS	NS
Hypoglycemia, aOR [95% CI]	NS	NS	NS
Seizure, aOR [95% CI]	NS	NS	NS
Need for resuscitation, aOR [95% CI]	NS	NS	NS

Abbreviations: PLIC: posterior limb of internal capsule, CI: confidence interval, aOR: adjusted odds ratio, NS: non-significant.

4. Discussion

This is a population-based cohort study of term neonates with NAIS born over an 11-year period in Central-Hungary. The main importance of this study is that a high proportion of infants had follow-up, performed between 18 months of age up to early school age, allowing for detailed documentation of the status of long-term neurodevelopmental outcome data. Longitudinal examination of neurological sequelae in patients with perinatal brain injury is particularly important because speech and other higher cognitive functions emerge later in childhood [27]. Importantly, in our study, almost half of the patients (43%) had normal outcome likely reflecting, among others, the potential of the developing brain for reorganization. Nevertheless, we have also demonstrated that main MCA stroke, multiple strokes, the involvement of the CST and infection/inflammation were independent predictors of adverse outcome.

In addition to allowing for the prompt diagnosis of NAIS, brain MRI scans also aid the clinician in the prognostication of long-term outcome based on stroke territory subtypes [15,28]. It has been generally accepted that the ability of early prognostication and parental counseling are of clinical relevance in patients with NAIS as early intervention strategies may aid in optimizing motor and mental development [29]. In agreement with previous observations [15], we have also found that the volume of territorial involvement is important in predicting adverse outcome [30,31] as seen in the cases of main branch MCA stroke and multiple strokes. Of note is that main branch MCA stroke was not only associated with neurological impairment in most of the affected patients (93%) but the rate of CP was especially high (87%) compared to patients with other stroke subtypes (8–19%). Nevertheless, all of these neonates had a large stroke with the involvement of the CST, the basal ganglia, the thalamus and the cerebral cortex. Therefore, we cannot conclude whether the large territorial involvement in itself, the involvement of certain specific anatomic regions or both are responsible for the development of CP/adverse outcome in these patients.

Involvement of the CST has been associated with poor outcome in general and CP in particular [15,17,18]. Interestingly, other studies have shown that the concomitant involvement of the cortical and subcortical structures, particularly the PLIC, the basal ganglia and the cerebral cortex predicted hemiplegia [10]. Therefore, to better investigate the role of CST involvement in the long-term outcome of NAIS, after excluding infants with main branch MCA stroke, we assessed the impact of injury of the CST at the level of PLIC with or without the involvement of cerebral peduncles. While controlling for other important clinical variables, involvement of the CST increased the overall risk for adverse outcome and CP/fine motor impairment. Nevertheless, 40% of the patients with CST involvement without a widespread stroke did not have motor deficit in the long-run and 25% of them only had fine motor impairment. These findings might be explained by the neuroplasticity of the developing brain following perinatal brain injury.

Studies have reported the rate of epilepsy to be in the 10–54% range in cases with NAIS [32–35] and we have found that the overall rate of epilepsy was 13% in our cohort. Our findings also indicate that the risk of developing epilepsy is higher with main branch MCA or multiple strokes and when there is clinical evidence of inflammation/infection. These findings support previous observations that the risk of developing epilepsy is higher when multiple vessels are involved or when more substantial tissue damage occurs [34]. Additionally, our observations that epilepsy co-occurred with other neurodevelopmental sequelae such as cognitive impairment and CP suggest that certain neurodevelopmental outcomes might share a common origin [15,36] and that epilepsy might limit plasticity of the brain during development [37,38].

Although infection has been recognized as a risk factor for neonatal or childhood stroke [39], to the best of our knowledge, this is the first

study that reports the presence of specific or non-specific inflammation as an independent predictor for adverse neurodevelopmental outcome, in particular CP, cognitive impairment and epilepsy in patients with NAIS. We speculate that the inflammation-associated release of cytokines, chemokines and other local factors might lead to the development of selective intracranial arteritis [40], increase the severity of the stroke [5] and/or affect brain development in the postnatal period.

There is no clear evidence whether arterial stenosis or vessel irregularity contributes to the development of cerebral infarction [41] or alternative mechanisms such as vasospasm [42] play a role in the development of NAIS in the presence of signs of infection. In our study, rates of cerebral arteriopathy were similar in neonates with and without evidence for inflammation/infection. While neonates with large areas of infarct/multiple strokes are at higher risk for poor outcome [30,34,43], in the present study, neonates with infection/inflammation did not have increased areas of infarct compared to those without infection/inflammation. Therefore, the unfavorable outcome in this subgroup of patients might not be attributed to the size of the stroke. As elevated CRP with or without culture positivity was an independent predictor of poor long-term neurodevelopmental outcome, it is tempting to speculate that patients, who respond to the tissue damage with enhanced inflammation, with or without an identifiable infectious source, might be at risk for more substantial tissue damage [44]. Clearly, further prospective studies are needed to delineate the association between NAIS and inflammation and adverse outcomes. Yet, it is reasonable to suggest exercising caution when inflammatory reaction is detected in this patient population during the acute phase of the disease process and initiating appropriate early interventions and a thorough follow-up in this patient population.

Finally, in line with previous findings [34,45], we have found that severe disability and the co-occurrence of impairment in all neurological domains were rare. Moreover, 43% of the patients had normal long-term neurodevelopmental outcome. As approximately 30–60% of patients with NAIS develop CP, current treatment has primarily focused on improving motor outcomes. Indeed, constraint induced movement therapy and transcranial magnetic stimulation have been shown to improve motor outcome [46]. Since central nervous system plasticity is likely the maximal in the neonatal period and during early infancy [47], early initiation of developmental support, environmental enrichment and parental advocacy are thought to be of great importance in ameliorating brain injury and enhancing the chances of white matter recovery [48].

Our study also has limitations to be considered. Firstly, the analysis conducted utilized in part retrospectively collected data and was performed on data obtained over a relatively long period of time, with its inherent disadvantages. Secondly, due to the uncommon diagnosis of NAIS, we collected data from all Neonatal Intensive Care Units in Central Hungary with somewhat diverse clinical management styles. Nevertheless, the relatively high level of harmonization by the universal health care system in Hungary might have attenuated some of the consequences of the multicenter nature of the data collection. Finally, some subgroups of important anatomical lesions were too small to draw statistical conclusions. As NAIS is a rare condition, thoroughly controlled data collection by regional or national registries utilizing an appropriate oversight structure remain the main avenues for statistically robust data collection, hypothesis generation and testing for treatment and determining outcome of neonates with this condition.

5. Conclusions

We found that close to half of the patients with NAIS had normal neurodevelopmental outcome. On the other hand, main MCA stroke, multiple strokes, the involvement of the CST and infection/inflammation were all independent predictors of adverse outcome, suggesting that the interplay between stroke territorial involvement and clinical risk factors might influence the evolution of the disease and long-term brain developmental processes.

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CRediT authorship contribution statement

E. Vojcek collected clinical and MRI data, arranged neurodevelopmental follow-up assessments of children with neonatal arterial ischemic stroke, performed statistical analysis conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. I. Seri conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. A. Jermendy was involved in in systematization of data and reviewed and revised the manuscript. R. Graf and M. Berenyi assessed most of the neurodevelopmental outcomes, collected data and reviewed and revised the manuscript. A. M. Laszlo performed the statistical analysis and reviewed and revised the manuscript. G. Rudas evaluated the MRI scans. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Clinical data of four of the patients included in this study were published earlier (Ideggyogy Sz. 2018. **71**:127-136).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] T.N. Raju, K.B. Nelson, D. Ferriero, J.K. Lynch, NICHD-NINDS Perinatal Stroke Workshop Participants, Ischemic perinatal stroke: summary of a workshop sponsored by the National Institute of Child Health and Human Development and the National Institute of Neurological Disorders and Stroke, Pediatrics. 120 (3) (2007) 609–616.

[2] Grunt S, Mazenauer L, Buerki SE, Boltshauser E, Mori AC, Datta AN, et al. Incidence and outcomes of symptomatic neonatal arterial ischemic stroke. Pediatrics 2015; 135(5):e1220-e8.

[3] M. Dunbar, A. Kirton, Perinatal stroke: mechanisms, management, and outcomes of early cerebrovascular brain injury, Lancet Child Adolesc Health. 2 (9) (2018) 666–676.

[4] P. Govaert, L. Ramenghi, R. Taal, L. de Vries, G. Deveber, Diagnosis of perinatal stroke I: definitions, differential diagnosis and registration, Acta Paediatr. 98 (10) (2009) 1556–1567.

[5] K.B. Nelson, J.K. Lynch, Stroke in newborn infants, Lancet Neurol. 3 (3) (2004) 150–158.

[6] R. Laugesaar, A. Kolk, T. Tomberg, T. Metsvaht, M. Lintrop, H. Varendi, et al., Acutely and retrospectively diagnosed perinatal stroke: a population-based study, Stroke. 38 (8) (2007) 2234–2240.

[7] S. Chabrier, B. Husson, M. Dinomais, P. Landrieu, S. NTT, New insights (and new interrogations) in perinatal arterial ischemic stroke, Thromb. Res. 127 (1) (2011) 13–22.

[8] K.B. Nelson, Perinatal ischemic stroke, Stroke. 38 (2007) 742–745.

[9] E. Mercuri, M. Rutherford, F. Cowan, J. Pennock, S. Counsell, M. Papadimitriou, et al., Early prognostic indicators of outcome in infants with neonatal cerebral

infarction: a clinical, electroencephalogram, and magnetic resonance imaging study, Pediatrics. 103 (1) (1999) 39–46.
[10] J.P. Boardman, V. Ganesan, M.A. Rutherford, D.E. Saunders, E. Mercuri, F. Cowan, Magnetic resonance image correlates of hemiparesis after neonatal and childhood middle cerebral artery stroke, Pediatrics. 115 (2) (2005) 321–326.
[11] A. Kolk, M. Ennok, R. Laugesaar, M.L. Kaldoja, T. Talvik, Long-term cognitive outcomes after perinatal stroke, Pediatr. Neurol. 44 (2) (2011) 101–109.
[12] P. Ilves, T. Tomberg, J. Kepler, R. Laugesaar, M.L. Kaldoja, K. Kepler, et al., Different plasticity patterns of language function in children with perinatal and childhood stroke, J. Child Neurol. 29 (6) (2014) 756–764.
[13] K.J. Bowers, G.A. Deveber, D.M. Ferriero, E.S. Roach, Z.S. Vexler, B.L. Maria, Cerebrovascular disease in children: recent advances in diagnosis and management, J. Child Neurol. 26 (9) (2011) 1074–1100.
[14] R. Westmacott, D. MacGregor, R. Askalan, G. de Veber, Late emergence of cognitive deficits after unilateral neonatal stroke, Stroke. 40 (6) (2009) 2012–2019.
[15] N. Wagenaar, M. Martinez-Biarge, N.E. van der Aa, I.C. van Haastert, F. Groenendaal, M.J.N.L. Benders, et al., Neurodevelopment after perinatal arterial ischemic stroke, Pediatrics. 142 (3) (2018), e20174164.
[16] E. Vojcek, M. Csécsei, E. Flach, G. Rudas, R. Gráf, E. Princzkel, Perinatal stroke - from symptoms to follow-up, Ideggyogy Sz. 71 (3–4) (2018) 127–136.
[17] A. Biswas, K. Mankad, M. Shroff, P. Hanagandi, P. Krishnan, Neuroimaging perspectives of perinatal arterial ischemic stroke, Pediatr. Neurol. 113 (2020) 56–65.
[18] B. Husson, L. Hertz-Pannier, C. Renaud, D. Allard, E. Presles, P. Landrieu, et al., Motor outcomes after neonatal arterial ischemic stroke related to early MRI data in a prospective study, Pediatrics. 126 (4) (2010) 912–918.
[19] M. Dinomais, L. Hertz-Pannier, S. Groeschel, S. Chabrier, M. Delion, B. Husson, et al., Long term motor function after neonatal stroke: lesion localization above all, Hum. Brain Mapp. 36 (12) (2015) 4793–4807.
[20] M.S. Scher, Electroencephalography of the newborn : Normal and abnormal features, in: Niedermeyer E, Lopes da Silva FH, Eds Electroencephalography, 5th ed, Lippincott Williams and Wilkins, Philadelphia, 2005, pp. 937–990.
[21] N. Bayley, BSID-II: Bayley Scales of Infant Development, Second edition, Hartcourt Brace & Company, San Antonio, 1993.
[22] O. Brunet, I. Lézine, D. Josse, al e. Brunet-Lézine révisé : échelle de développement psychomoteur de la première enfance : manuel BLR-C, Issy-Les-Moulineaux (France), Etablissements d'Applications Psychotechniques, 1997.
[23] G.H. Roid, Stanford Binet Intelligence Scales, 5th ed., Riverside Publishing, Itasca, IL, 2003.
[24] R. Palisano, P. Rosenbaum, S. Walter, D. Russell, E. Wood, B. Galuppi, Development and reliability of a system to classify gross motor function in children with cerebral palsy, Dev. Med. Child Neurol. 39 (1997) 4.
[25] A.T. Berg, S.F. Berkovic, M.J. Brodie, J. Buchhalter, J.H. Cross, Boas W. van Emde, et al., Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE commission on classification and terminology, 2005–2009, Epilepsia. 51 (4) (2010) 676–685.
[26] L. Bender, Psychopathology of Children with Organic Brain Disorders, Charles C Thomas Publisher, Ltd, 1956.
[27] A. Kirton, G. Deveber, Life after perinatal stroke, Stroke. 44 (11) (2013) 3265–3271.
[28] B. Husson, L. Hertz-Pannier, C. Renaud, D. Allard, E. Presles, P. Landrieu, et al., Motor outcomes after neonatal arterial ischemic stroke related to early MRI data in a prospective study, Pediatrics 126 (4) (2010) 912–918.
[29] I. Novak, C. Morgan, L. Adde, J. Blackman, R.N. Boyd, J. Brunstrom-Hernandez, et al., Early, accurate diagnosis and early intervention in cerebral palsy: advances in diagnosis and treatment, JAMA Pediatr. 171 (9) (2017) 897–907.
[30] Mackay MT, Slavova N, Pastore-Wapp M, Grunt S, Stojanovski B, Donath S, et al. Pediatric ASPECTS predicts outcomes following acute symptomatic neonatal arterial stroke. Neurology. 2020;94:e1259-e70.
[31] V. Ganesan, M. Prengler, M.A. McShane, M.A. Wade, F.J. Kirkham, Investigation of risk factors in children with arterial ischemic stroke, Ann. Neurol. 53 (2003) 167–173.
[32] L.L. Lehman, M.J. Rivkin, Perinatal arterial ischemic stroke: presentation, risk factors, evaluation, and outcome, Pediatr. Neurol. 51 (6) (2014) 760–768.
[33] R.A. Shellhaas, T. Chang, C.J. Wusthoff, et al., Treatment duration after acute symptomatic seizures in neonates: a multicenter cohort study, J. Pediatr. 181 (2017) 298–301.
[34] A. Suppiej, M. Mastrangelo, L. Mastella, P. Accorsi, L. Grazian, G. Casara, et al., Pediatric epilepsy following neonatal seizures symptomatic of stroke, Brain and Development 38 (1) (2016) 27–31.
[35] C.K. Fox, M.T. Mackay, M.M. Dowling, P. Pergami, L. Titomanlio, G. Deveber, et al., Prolonged or recurrent acute seizures after pediatric arterial ischemic stroke are associated with increasing epilepsy risk, Dev. Med. Child Neurol. 59 (1) (2017) 38–44.
[36] K.L. Mueller, J.B. Tomblin, Examining the comorbidity of language disorders and ADHD, Top. Lang. Disord. 32 (3) (2012) 228–246.
[37] A.O. Ballantyne, A.M. Spilkin, J. Hesselink, D.A. Trauner, Plasticity in the devloping brain: intellectual, language and academic functions in children with ischemic perinatal stroke, Brain 131 (11) (2008) 2975–2985.
[38] K.C. Fitzgerald, L.S. Williams, B.P. Garg, M.R. Golomb, Epilepsy in children with delayed presentation of perinatal stroke, J. Child Neurol. 22 (11) (2007) 1274–1280.
[39] H.J. Fullerton, N.K. Hills, M.S. Elkind, M.M. Dowling, M. Wintermark, C.A. Glaser, et al., Infection, vaccination, and childhood arterial ischemic stroke: results of the VIPS study, Neurology. 85 (17) (2015) 1459–1466.
[40] Guiraut C, Cauchon N, Lepage M, Sébire G. Perinatal arterial ischemic stroke,is associated to materno-fetal immuno activation and intracranial arteritis. Int. J. Mol. Sci. 2016;17(12):E1980.
[41] W. Jan, R.A. Zimmerman, L.T. Bilaniuk, J.V. Hunter, E.M. Simon, J. Haselgrove, Diffusion-weighted imaging in acute bacterial meningitisin infancy, Neuroradiology. 45 (2003) 634–639.
[42] E.L. Lyons, N.E. Leeds, The angiographic demonstration of arterial vascular disease in purulent meningitis. Report of a case, Radiology 88 (1967) 935–938.
[43] M. Dunbar, H. Shah, S. Shinde, J. Vayalumkal, O.G. Vanderkooi, X.C. Wei, et al., Stroke in pediatric bacterial meningitis: population-based epidemiology, Pediatr. Neurol. 89 (2018) 11–18.
[44] H.M. den Hertog, J.A. van Rossum, H.B. van der Worp, H.M.A. van Gemert, R. de Jonge, P.J. Koudstaal, et al., C-reactive protein in the very early phase of acute ischemic stroke: association with poor outcome and death, J. Neurol. 256 (2009) 2003.
[45] S. Chabrier, E. Peyric, L. Drutel, J. Deron, M. Kossorotoff, M. Dinomais, et al., Multimodal outcome at 7 years of age after neonatal arterial ischemic stroke, J. Pediatr. 172 (2016) 156–161.
[46] A. Kirton, J. Andersen, M. Herrero, A. Nettel-Aguirre, L. Carsolio, O. Damji, et al., Brain stimulation and constraint for perinatal stroke hemiparesis: the PLASTIC CHAMPS trial, Neurology. 86 (18) (2016) 1659–1667.
[47] Craig BT, Hilderley A, Kinney-Lang E, Long X, Carlson HL, Kirton A. Developmental neuroplasticity of the white matter connectome in children with perinatal stroke. Neurology. 2020;95(18):e2476-e86.
[48] Forbes TA, Goldstein EZ, Dupree JL, Jablonska B, Scafidi J, Adams KL, et al. Environmental enrichment ameliorates perinatal brain injury and promotes functional white matter recovery. Nat. Commun.. 2020;11(964).