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PERINATAL STROKE

PhD thesis

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List of Abbreviations:

AIS: arterial ischemic stroke

ACA: anterior cerebral artery

BSID-II: Bayley Scales of Infant Development, Second Edition,

CI: confidence interval,

CP: cerebral palsy,

CST: corticospinal tract,

CSVT: cerebral sinovenous thrombosis,

DWI: diffusion-weighted imaging,

EEG: electroencephalogram,

GDM: gestational diabetes mellitus,

HIE: hypoxic ischemic encephalopathy,

HS: hemorrhagic stroke,

IQR: interquartile range,

MCA: middle cerebral artery,

MRI: magnetic resonance imaging,

MTHFR: methylenetetrahydrofolate reductase,

NICUs: Neonatal Intensive Care Units,

OR: odds ratio,

PAIS: perinatal arterial ischemic stroke,

PCA: posterior cerebral artery,

PFA: patent foramen ovale,

PHS: perinatal hemorrhagic stroke,

PLIC: posterior limb of the internal capsule,

PPS: presumed perinatal stroke,

PROM: premature rupture of membranes,

SD: standard deviation,

SGA: small for gestational age,

T1W: T1-weighted,

T2W: T2-weighted,

VP: ventriculoperitoneal

1. Introduction

The pregnant woman and her fetus have risk factors that shift the balance of 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 presenting acutely as arterial ischemic stroke (AIS), hemorrhagic stroke (HS) or cerebral sinovenous thrombosis (CSVT). In *ischemic stroke*, vessels are most commonly occluded by thrombosis or embolism. Occlusion can also be caused by direct trauma, compression, spasm or obliteration by an inflammatory process [2]. *Hemorrhagic stroke* (parenchymal hemorrhage) is either a primary hemorrhagic stroke with or without a suspected underlying etiology such as vascular malformations, bleeding diathesis, arteriovenous malformation or cavernoma, or secondary hemorrhagic stroke resulting from hemorrhagic transformation of a primary ischemic injury [3]. Finally, *in cases with cerebral sinovenous thrombosis (CSVT)*, a portion or the entire sinus venosus may be involved in the thrombotic process.

Perinatal stroke has been defined as an acute cerebral tissue damage occurring between 20 weeks of gestation and 28 days after delivery and carrying the risk of chronic neurological sequelae.[1] Based on the *time of presentation*, it is further classified into antenatal (fetal), neonatal and presumed perinatal stroke (PPS). Presumed perinatal stroke is defined as an infant (term equivalent age + 4 weeks) presenting with imaging findings suggestive of longstanding stroke without previous fetal or neonatal symptoms [1]. Presumed perinatal stroke can be further divided into presumed perinatal arterial ischemic stroke, perinatal venous infarction and presumed perinatal hemorrhagic stroke. [1]

The estimated incidence of perinatal stroke is approximately one in 1000 live births.[4] The incidence of perinatal stroke is the second highest after that of the ischemic stroke in the elderly most likely because both the mother and the fetus have transient risk factors predisposing the newborn to a prothrombotic state. [1] However, the true incidence is likely higher due to the lack of routine use of magnetic resonance imaging (MRI) in most neonatal units. In addition, neonates with CSVT are often also not classified as having a perinatal stroke.[5, 6]

As perinatal stroke accounts for approximately 30% of children affected with hemiparetic cerebral palsy (CP), the condition is an important cause of CP in childhood.[7, 8] In addition, survivors may also have cognitive [9] and language impairment,[10] epilepsy,[11] and/or behavioral disorders often manifesting only at school age.[12]

A better understanding of the relationship among the risk factors, findings of imaging studies and long-term neurodevelopmental outcome has been suggested to improve the accuracy and timing of the diagnosis and treatment of perinatal stroke.[11] Moreover, the pathophysiology of perinatal stroke is likely different in newborns with different risk factors.[13] Since long-term, risk factor-specific outcomes of this injury have not yet been described, our aim was to *investigate the long-term neurodevelopmental outcomes of different clinical subgroups of newborns with different forms of perinatal stroke and assess the impact of the findings of MRI, electroencephalography (EEG) and clinical risk factors on different aspects of neurodevelopment.*

1.1 Risk factors of perinatal stroke

Potential observed risk factors may act synergistically to increase the risk of perinatal stroke, and in most cases the coexistence of multiple risk factors contribute to the formation of thromboemboli. Therefore, these factors by themselves cannot be inferred as definitive etiologies of perinatal stroke. Potential risk factors for perinatal stroke comprise maternal, fetal, and placental conditions as described by Raju et al. and Nelson and Lynch [1, 14] (Table 1).

Table 1. Risk factors for perinatal stroke [1, 14, 15]

Maternal risk factors during pregnancy:

Prothrombic state during pregnancy

Autoimmune disorders

Drug abuse

Preeclampsia

Infection/Prolonged rupture of membranes

Inherited or acquired thrombophilias

Infertility and its treatment

Labor and delivery complications

Fetal/Neonatal factors:

Inherited or acquired thrombophilias

Twin-to-twin transfusion

Central nervous system/systemic infection

Perinatal asphyxia

Congenital heart disease

Patent ductus arteriosus

Extracorporeal membrane oxygenation

Open heart surgery/balloon atrial septostomy

Polycythemia

Catheter-related complications

Vascular maldevelopment/Vasculopathy

Miscellaneous factors:

Male gender

Race and ethnicity

Dehydration

The proinflammatory status of pregnancy also increases the risk of clot formation. During pregnancy there is an increase in thrombin generation as protein C, von Willebrand factor, factor VIII, factor V, and fibrinogen concentrations are high while the endogenous anticoagulant protein S and activated protein C ratios are reduced. Plasminogen activator inhibitor 1 and 2 are also increased resulting in impaired fibrinolysis [16]. Infections can exacerbate these tendencies because the function of protein C and antithrombin III are impaired while the endothelial injury and release of inflammatory cytokines result in decreased thrombomodulin production and accumulation of tissue factor [14]. Guiraut et al observed that, in newborn pups, the prothrombotic stress caused by *E. coli* lipopolysaccharide leads to focal intracranial arteritis selectively in the carotid tree. This process, in the setting of the perinatal pro-coagulation state, resulted in the development of stroke [17].

Inherited or acquired thrombophilia may predispose to thrombosis. Different forms of genetic thrombophilia such as increased lipoprotein(a), factor V G1691A mutation, prothrombin G20210A variant, methylenetetrahydrofolate reductase (MTHFR) T677T genotype, antithrombin III, protein C deficiency, protein S deficiency and acquired forms of thrombophilia such as antiphospholipid antibodies and lupus anticoagulant were investigated to examine their effect on the occurrence of perinatal ischemic stroke [18]. While Gunther et al suggest screening for prothrombotic risk factors in children suffering vascular accidents during the neonatal period [18], others found that presence of thrombophilia in children with ischemic perinatal stroke is rare and is comparable to that in the normal population, and therefore does not indicate routine testing in childhood [19]. Genetic risk factors of thrombophilia and combined prothrombotic disorders were proved to be important risk factors for manifestation of ischemic strokes in children with underlying cardiac disorders [20]. The potential interaction between fetal or maternal thrombophilia and other diseases or major risk factors underly the multifactorial etiology of symptomatic ischemic stroke in neonates.

Thromboembolism can originate from intracranial or extracranial vessels, or the heart, or placenta [14]. The patency of the foramen ovale and the right-to-left shunting through the fetal ductus arteriosus enables the thrombus to pass into the arterial system and cause injury in the fetal brain or other organs. Therefore, macroscopic and microscopic examinations of the placenta and the umbilical cord should be performed ideally [15].

Both congenital and acquired heart disease predispose to perinatal and/or pediatric stroke. Not unexpectedly, cyanotic and/or complex congenital heart diseases appear to increase the risk most. The prevalence of cardiac disease was found in 10%-31% of neonates and children with stroke depending on the inclusion of infants with isolated patent foramen ovale (PFO). [21] It is also important to note that AIS is more likely to transform into hemorrhagic stroke in children with cardiac disease. [21] Cardiopulmonary bypass, hypoperfusion during prolonged surgery, balloon atrial septostomy and reoperation also increase the risk of stroke. Syndromes of cardiac disease are often accompanied by cerebral vasculopathies [21].

Intracranial vascular lesions (arteriovenous malformations, cavernous malformations, or aneurysm) account for approximately half of the cases with pediatric stroke [22]. Additionally, the presence of mutations in collagen 4A1 gene, a major structural protein of the developing cerebral vasculature, may result in hemorrhagic stroke especially in the presence of delivery complications [23]. Possible birth injury secondary to vacuum extraction, prolonged second stage labor and shoulder dystocia has been suggested to result in intracranial arterial injury in neonates [24] although this notion has been disputed.[25]

Additional maternal risk factors include gestational hypertension and/or preeclampsia [26].

The fetal asphyxia is an additional risk factor of perinatal stroke. Animal studies have shown that hypovolemic and hypoxic-ischemic states are associated with dilation of the ductus venosus and changes in umbilical venous pressure resulting in increased blood flow in the ductus venosus, the left heart and ultimately, the brain.[27, 28] Birth asphyxia and hypoxic-ischemic encephalopathy also predict moderate or severe cerebral palsy in perinatal stroke [29]. While hypoxic-ischemic encephalopathy and perinatal stroke are two distinct entities, a recent study using multivariate analysis found that an

Apgar score of <7 at 5 minutes was associated with the development of perinatal AIS [25]. Nevertheless, as a general consensus [2], border zone injury (watershed infarction) resulting from partial asphyxia or systemic hypotension is not regarded as stroke [2].

1.2 Clinical symptoms

According to the International Pediatric Stroke Study [30], 87% of the neonates with ischemic perinatal stroke present in the first postnatal week, in general and during 12-72 hours after delivery in particular. The most common presenting clinical signs are seizures (69-90%) and nonspecific neurologic symptoms (63%) such as abnormal tone (38%) and altered level of consciousness (irritability/lethargy) (39%). [30] Since in most cases AIS affects the middle cerebral artery (MCA), focal neurological signs present involving movement and sensation of the upper extremities and face but not the lower extremities. [31] Bilateral lesions may cause mild quadriparesis with exaggerated primitive reflexes. [31] One third of the patients demonstrate focal neurologic signs, mainly lateralizing hemiparesis. [30] Systemic symptoms also may occur including respiratory (26%) and feeding (24%) difficulties, and, with the involvement of the parietal or occipital lobes, sensory deficits may also appear. [30]

Eighty-one percent of *neonates with CSVT* present on the day of birth or within the first week after delivery and in 71% of the cases seizures occur. [32] Diffuse neurologic signs such as altered level of consciousness and jitteriness are the second most common clinical signs (58%), whereas focal signs including hemiparesis and cranial nerve palsy take place in one-third of the cases. [32]

Clinical signs of perinatal hemorrhagic stroke present during the first 28 days of life in 94% of the subjects. Common clinical presentations consist of seizures (67%) and/or encephalopathy (83%) and/or hypotonia (42%). [3] In half and 16% of the affected patients Apgar scores are less than 5 at 1 and 5 minutes, respectively [3].

The most common symptom of presumed perinatal stroke is hemiparesis presenting between 4 and 8 months' of age after an unremarkable neonatal course and with MRI findings consistent with a stroke of perinatal origin. [33] The most common complaint is asymmetry in reaching for and grasping objects with early handedness. Rarely, presumed perinatal stroke only is suspected at school age presenting with learning disability, cognitive delay, visual field defect or epilepsy [33].

1.3 Diagnosis

As a first step, cranial ultrasound is used in neonatal intensive care units to screen for cranial abnormalities in preterm or term neonates. Cranial ultrasound scans within the first three postnatal days have a sensitivity of 68% in infants with cerebral ischemic stroke, while this percentage increases to 87% when scans are performed after day #4. [34] The typical appearance is a wedge-shaped lesion of increased echogenicity involving the cortex and white-matter of the affected arterial territory. [35] Yet, cranial ultrasound scan does miss small, cortical infarcts involving the periphery of the brain, infarct affecting the posterior circulation and cerebral sinovenous thrombosis of the deep medullary veins [35]. However, cranial ultrasonography is useful in infants with perinatal hemorrhagic stroke since the hemorrhage can be visualized as an increased echogenicity. [35] Doppler studies in patients with infarcts involving the MCA may show transiently reduced flow and pulsatility in the affected artery within the first 24 hours after the development of stroke [36].

The extent and vascular territorial involvement in AIS, the presence and location of CSVT and associated infarction and hemorrhage is most reliably confirmed by MRI. In addition, MRI also detects small and early infarcts. At the very least, the MRI study should include axial diffusion-weighted imaging (DWI), apparent diffusion coefficient scan (ADCs), susceptibility-weighted imaging, and magnetic resonance angiography (MRA). These modalities a list can be completed using T2- and T1-weighted sequences [37]. Diffusion-weighted imaging (DWI) is used to visualize cytotoxic edema in early ischemic infarction, especially after the first 2 days of clinical presentation while the sensitivity of DWI declines after 5 days of the initial presentation. [38] During the first week after the onset of ischemic stroke, conventional T2-weighted images reveal a high-signal intensity in the affected cortical gray and white matter. This persistent hyperintensity is called the „*T2 shine through*”. [37] From one week to one month after the onset of ischemic stroke, organization of the infarct takes place with neovascularization, myelin breakdown, and gliosis. [37] The combination of petechial hemorrhage, lipid laden microglia, high protein content, and manganese accumulation in the reactive astrocytes leads to a high T1 signal in the cortex (“*cortical highlighting*”) with corresponding low-signal intensity on T2W imaging. [37] After 1-2 months, the

infarcted area evolves into an area of tissue loss [38] leading to the formation of a smooth-walled fluid filled cavity, the “*porencephalic cyst*”.[37] Magnetic resonance angiography of the head and neck is also useful in the detection of abnormalities and/or anatomical variants of the neurovascular system in patients with perinatal stroke, as vasculopathy is the underlying pathology in 20%-35% of the cases. [39]. MR imaging is also used for the detection of parenchymal hemorrhage particularly to detect concomitant ischemic changes. These ischemic changes are important predictors of neurodevelopmental outcome.[35] Susceptibility-weighted imaging can highlight petechial hemorrhage along with added MR angiography and MR venography.[35]

In addition to allowing for the prompt diagnosis of perinatal stroke, MRI scans may also aid the clinician in the prognostication of long-term neurodevelopmental outcome by determining the stroke territory-subtypes. The site, extent and location affected by the stroke can be used to predict adverse neurodevelopmental outcome, particularly motor outcome. The involvement of the corticospinal tract is associated with poor outcome and the development of cerebral palsy. [37, 40, 41] Interestingly and perhaps not quite unexpectedly, studies have found that concomitant involvement of the cortical and subcortical structures, particularly the posterior limb of the internal capsule (PLIC), the basal ganglia and the cerebral cortex predicts hemiplegia [8]. Studies have also found that main branch MCA stroke results in 100% adverse neurodevelopmental outcome [41]. Indeed, the size of the brain volume involved in itself seems to be important in predicting normal vs. adverse outcome. [42, 43] Adverse cognitive outcome and behavioral problems have also been associated with combined involvement of the basal ganglia and thalami.[41] Strokes involving the main and posterior branches of MCA and the PCA have been shown to be associated with an increased the risk for cognitive delay.[41] Finally, the risk of developing epilepsy is higher when multiple vessels are involved or when more substantial tissue damage occurs, [32] In addition, post-neonatal epilepsy is associated with the involvement of the cerebral peduncle and bilateral lesions [41].

Based on the findings of imaging studies, the majority of perinatal ischemic strokes are unilateral, occurring in the left hemisphere within the MCA territory. [26] According to the findings of the International Pediatric Stroke Study, strokes are more commonly left-sided (51%), while bilateral lesions are only observed in 24% of the patients [26].

Similarly, Lee et al. found that 87% of strokes are unilateral. [44] Again, the he majority of the strokes involved on the left side (53%), and MCA territory was affected in 74% of the patients [44]. A reasonable explanation for the more frequent left sided strokes might be the differences in the flow pattern of the ductus arteriosus or the more direct route from the left common carotid. In perinatal hemorrhagic stroke, the most involved lobe is the temporal lobe affecting up to one-third of the subjects [45]. In CSVT, the most commonly obstructed sinus is the transverse sinus [46]. Yet, in one quarter of the patients, multiple sinuses are obstructed. Complete obstruction of the great vein of Galen and basal veins leads to rapid death which might be one of the explanations for the unfavorable outcome of CSVT [46].

The location of cerebral infarction is also affected by the level of maturity. In term neonates, occlusions frequently occur in the cortical branches (59%), while preterm babies more common present with infarcts in the lenticulostriate branches (39%) [47, 48].

While computed tomography (CT) is sensitive in diagnosing perinatal hemorrhagic stroke, it has low sensitivity for detecting small infarcts, particularly posterior fossa ischemic strokes, and is also associated with radiation exposure. Therefore, CT is no longer recommended for routine imaging of patients suspected to have perinatal stroke. [37]

Further diagnostic studies include EEG preferably during the first 24 hours as most neonates with stroke develop seizures early between 12 and 72 hours after delivery, seizures are often subclinical or might not be recognized due to the associated cardiorespiratory deterioration. Seizures might also affect the developing brain and be linked to lower intellectual and language performance.[49] Therefore, initiating prompt antiepileptic treatment in these patients is of importance. Finally, EEG might help predicting later neurodevelopmental outcome. For instance, a study with small number of patients found that infants with abnormal EEG background activity later developed hemiplegia [50]. Others described that prolonged and recurrent seizures after arterial ischemic stroke were associated with increasing risk of epilepsy [51]. Finally, slower recovery of background activity on ipsilesional amplitude-integrated EEG along with increased regional cerebral tissue oxygen saturation asymmetry between hemispheres

on NIRS was associated with an increased risk for cognitive deficit [52].

Since CHD is a known risk factor for stroke in the neonate [53, 54], it is clearly reasonable to perform a screening echocardiography in patients with perinatal AIS. Infants with congenital heart disease face multiple risk factors for stroke including right-to-left shunting and cardioembolism as well as multiple cardiac interventions. [54] Due to the advances in the management of neonates with congenital or acquired heart disease, mortality of neonates undergoing heart surgery has remarkably declined [55]. In addition, state-of-the art cardiac and intensive care allows more children to survive even with critical cardiac diseases. [56] As a consequence, motor and global developmental delay occurs more frequently in infants with CHD undergone complex cardiac and surgical care [57], and this has placed an increased burden on society in general and the families and patients in particular.

Assessment of the placenta is an important part of understanding the etiology and pathophysiology of perinatal stroke. However, due to the lack of routine placental histological testing and the difficulties of obtaining placental tissue hours or days after delivery once symptomatic perinatal stroke has been diagnosed in a neonate, findings of studying the placenta is frequently a “luxury” we have at our disposal. [58]

Laboratory investigation should include a complete blood count, serum concentrations of blood glucose, calcium, and electrolytes, and testing for inborn errors of metabolism. Examination of the cerebrospinal fluid, blood and cerebrospinal fluid cultures, and investigation of the urine for toxic substances are mandatory.

Performing routine coagulation studies is controversial. Recent studies suggest that patients with perinatal AIS demonstrate rates of prothrombotic disorders comparable to those in the general population [19]. Therefore, routine testing for thrombophilia in patients with perinatal stroke have been discouraged. However, in conditions other than AIS (e.g., cerebral sinovenous thrombosis) or when other major risk factors such as CDH may act synergistically to increase the risk of perinatal stroke, performance of coagulation studies are considered appropriate. On the other hand, Luciani et al found that out of 48 patients with persistent neurological disabilities presumably secondary to perinatal stroke, 22 (45.8%) had inherited thrombophilia. [59] These findings, although indirectly, suggest a role of inherited thrombophilia in the pathogenesis of perinatal

stroke [59]. In neonates with CHD, genetic and acquired thrombophilias (prothrombotic disorders) have also been reported at a greater frequency [21]. In children with a single prothrombotic disorder the odds ratio for AIS is increased by 1.5-9 times and 12-fold in children with two or more prothrombotic abnormalities. In summary, more research is required to assess the role of thrombophilia in primary stroke prevention in children with cardiac disease [21].

1.4 Treatment

In the acute phase, treatment of perinatal stroke is largely supportive. Assurance of adequate oxygenation and ventilation, correction of hypoglycemia, hypocalcemia, and other electrolyte disturbances and metabolic disorders as well as treatment of infections are the main goals of treatment. Seizures are treated with anticonvulsant medications and early cessation of antiepileptic treatment is recommended once the patient is symptom free. For neonates with AIS and documented cardioembolic source, the American College of Chest Physicians (ACCP) 2012 guidelines suggest the use of anticoagulation therapy with unfractionated heparin or LMWH. The ACCP guidelines also recommend the use of anticoagulant or aspirin therapy for neonates with recurrent AIS [60]. The American Heart Association (AHA) guidelines suggest the use of LMWH or unfractionated heparin for neonates with severe thrombophilic disorders or multiple cerebral or systemic emboli [61]. There is no randomized controlled trial on the use of thrombolytic therapy and the AHA guidelines recommend the avoidance of thrombolytic agents in neonates [61]. For neonates presenting with CSVT without significant intracerebral hemorrhage, the ACCP guidelines recommend to anticoagulate the patient with unfractionated heparin or LMWH and then use LMWH for a minimum of six weeks but not longer than three months [60]. Recanalization should be assessed at six weeks after treatment and anticoagulants should be stopped if recanalization is complete [60]. If recanalization is incomplete, anticoagulation therapy should be continued for a total treatment of three months [60]. The AHA guidelines suggest anticoagulation with LMWH or unfractionated heparin for neonates with clinical or radiologic evidence of progressing CSVT [61]. For neonates with

intracerebral hemorrhage, the AHA guidelines recommend correction of low platelet counts, replacement of deficient coagulation factors, administration of vitamin K in vitamin K-dependent coagulation disorders and placement of a ventricular drain for patients who develop hydrocephalus [61].

Early introduction of physical, occupational and speech therapy might help to achieve the best outcome possible after perinatal stroke. Constraint-induced movement therapy might be useful in children under 18 months of age with unilateral cerebral palsy [62, 63]. Gait training using treadmills, ramps and the ground has also been recommended in children under two years of age with perinatal stroke [64]. The use of transcranial magnetic stimulation appears to be safe in pediatric stroke and may improve hand function [65]. The effect of repetitive transcranial magnetic stimulation and constraint-induced movement therapy was studied in the PLASTIC CHAMPS trial and Class II evidence obtained from the trial indicates that combined use of rTMS and CIMT enhances functional motor gains in children with stroke-induced hemiparetic cerebral palsy [66]. In rodent models with AIS, therapeutic hypothermia decreased infarct size and improved outcome [67]. In neonates with perinatal stroke, therapeutic hypothermia seems to decrease the risk of neonatal seizures [68]. Treatment with erythropoietin in animal models of stroke decreased cerebral volume loss, increased neurogenesis and oligodendrogenesis and improved outcome [69, 70].

1.5 Long-term outcome

Long-term outcome of perinatal ischemic stroke varies significantly among the studies on the subject. This is most likely due to the differences in the assessment tools and the duration of follow-up. The presence of neurologic deficits have been documented in 49-75% of stroke- survivors and sensorimotor deficits are the most common findings [15]. Motor deficit occurs in 48-59% of neonates. The concomitant involvement of the PLIC, the basal ganglia (lenticulostriate vessels) the cerebral peduncles seen on early DWI are associated with the development of hemiplegia [8]. Involvement of descending corticospinal tract also results in hemiparesis as a long-term consequence [37, 40]. Language delay and behavioral disorders occur at 21% and 11% of children with perinatal ischemic stroke, respectively [44]. Diagnosed behavioral disorders include attention problems, hyperactivity, or behavioral problems. Children with perinatal stroke tend to grow into their deficits therefore long-term follow-up preferably until adolescence is suggested. Indeed, while children with perinatal ischemic stroke do not differ significantly from their unaffected peers at preschool age, 69% of them show a significant reduction in one or more IQ index-measures at school age [71]. Between 38% to 46% of neonates with AIS develop epilepsy. Presence of an infarct on prenatal ultrasonography and family history of epilepsy are significantly associated with seizures after 6 months of age [72]. In addition, of seventy patients with AIS, 15% developed epilepsy and 55% of those with epilepsy had focal epilepsy with or without secondary generalization while 45% had infantile spasms [73]. Remote seizures and epilepsy are associated with the involvement of cortex and basal ganglia in the stroke. It also appears that the younger the brain when affected by the stroke, the higher is the risk of developing epilepsy therefore epileptogenesis is more likely to develop at an early age [75]. However, epilepsy seems to decrease over time and in many cases with stroke epilepsy resolves with advancing age. [73] The risk of developing epilepsy is higher when the right MCA or multiple territories are involved [74]. Also, patients that develop prolonged and recurrent acute seizures after AIS has an increased the risk of epilepsy [75]. Mortality rate of neonatal AIS is 0.16 per 100,000 live births [25] and recurrence of stroke occurs in 2% of neonates mainly with CHD, arteriopathy or prothrombotic disorders [76].

The mortality rate after neonatal CSVT is much higher and varies between 2% to 24% [79]. Neurologic deficits (epilepsy, cerebral palsy and cognitive impairment) are reported to occur in 10% to 80% of the affected patients [79]. Although 21% to 82% of the surviving patients have been reported as normal, the impact of associated risk factors as well as the wide range of the duration of follow-up, lack of consistency in the assessment tools used explain the uncertainty surrounding the true range of normal development in children with perinatal stroke [77].

Mortality rate of perinatal hemorrhagic stroke has been reported at 4% without any single recurrent case [3]. As for long-term morbidity, sensorimotor deficit, impairment of language function and epilepsy were described in 42% of the survivors [3].

Finally, parents of children with perinatal stroke are at an increased risk to develop stress and anxiety. Mothers of children with mild conditions are indistinguishable from controls. However, mothers of children with moderate-to-severe conditions are at risk for depression, marital dissatisfaction, decreased quality of life, and impaired family functioning. To address the feeling of guilt and anxiety is one of the major goals in the care of the mother and the family [78].

2. Aims

The overall objective of this PhD thesis was to design a population-based study, involving all patients with acute perinatal stroke confirmed by MRI born over an 11-year period between 2007 and 2017 in Central-Hungary. The design of the population-based study was based on the findings of a case-series on acute perinatal stroke [79]. Our primary aim was to assess the incidence of acute perinatal stroke in Hungary and compare it to the data available in an international database. We also set out to investigate the clinical risk factors, diagnostic findings and long-term neurodevelopmental outcome of *different clinical subgroups of newborns with perinatal stroke* and describe the possible associations among the clinical presentation, potential clinical risk factors, magnetic resonance imaging (MRI) and electroencephalogram findings and long-term neurodevelopmental outcome. In our analysis, we specifically focused on neonates with PAIS, CSVT, PHS, in utero stroke, and we studied neonates with CHD *and* perinatal stroke as well as late preterm neonates with perinatal stroke. However, due to the space constraints and other limitations described for a PhD thesis, have we mainly focused on the findings in neonates with PAIS, PHS and CHD *and* perinatal stroke. To ensure patient homogeneity, we have divided the patient population into these categories and studied and described them accordingly. Also, here we do not address the question of *presumed perinatal stroke*, since patients with this condition often do not present with commonly recognizable clinical symptoms. Therefore, population-based studies on *presumed perinatal stroke* have been criticized for the likely failure to include all patients with this condition. Finally, we also provide a detailed review of the literature on perinatal stroke in general and its subtypes in particular. We have also included a proposed management guideline on the diagnosis and management strategies of acute perinatal stroke.

The specific aims were as follows:

2.1 To analyze the clinical characteristics of term neonates with the diagnosis of perinatal stroke in a case-series (patient data obtained from the database of the Neonatal Intensive Care Unit of Szent János Hospital for the years spanning from 2006 to 2017)

With a longitudinal study on patient data obtained for the years from 2006 to 2017, we analyzed the clinical presentation, imaging methods, etiology and the clinical relevance of perinatal stroke in a case-series. To assess the long-term neurodevelopmental outcome of patients with perinatal stroke, we systematically carried out a neurodevelopmental follow-up study of these patients at 2 years of age or later.

2.2 To assess the long-term neurodevelopmental outcome of term neonates diagnosed with PAIS and investigate the associations among stroke territorial involvement on MRI, clinical risk factors and neurodevelopmental outcomes

Since acute PAIS is the most frequent type of perinatal stroke with an incidence of 1 in 3000 live births, first we focused on term neonates affected by PAIS. To minimize patient heterogeneity, we excluded late preterm neonates from this analysis. Long-term follow-up studies of patients with PAIS are limited, and population-based, longitudinal follow-up studies are lacking. Therefore, we designed a population-based study, involving all patients diagnosed with PAIS born over an 11-year period between 2007 and 2017 in Central-Hungary. Lesion characteristics on MRI have been shown to be helpful in predicting outcome in patients with PAIS and other stroke types (e.g., size, location of lesion, loss of PLIC signal on DWI, etc). [41, 42] Therefore, our primary aim was to investigate the clinical risk factors, diagnostic findings and long-term neurodevelopmental outcomes *of newborns with PAIS*. Our secondary aim was to describe the possible associations among clinical presentation, potential clinical risk factors, magnetic

resonance imaging (MRI) and electroencephalogram findings and long-term neurodevelopmental outcome.

2.3 Long-term neurodevelopmental outcome of term neonates with perinatal hemorrhagic stroke: a population-based study

Perinatal hemorrhagic stroke results in significant long-term neurodevelopmental disability in the majority of the survivors. A better understanding of the relationship among the risk factors, findings of imaging studies and long-term neurodevelopmental outcome has been suggested to improve the accuracy and timing of the diagnosis and treatment of PHS.[11] Since appropriately powered, population-based studies investigating the long-term outcome of patients with this condition are limited, we designed a population-based study, involving all patients with PHS born over an 11-year period between 2007 and 2017 in Central-Hungary. Our aim was to investigate the long-term neurodevelopmental outcome of newborns with PHS and assess the impact of the brain territorial involvement and clinical risk factors on the long-term outcome in this patient population.

2.4 Mortality and long-term outcome of neonates with congenital heart disease and acute perinatal stroke: a population-based case-control study

As mentioned earlier, little is known about the long-term neurodevelopmental outcome of neonates with perinatal stroke [30, 80]. This is especially true for neonates with *perinatal stroke and CHD* [2]. Therefore, we conducted a population-based case-control study enrolling 28 term and near-term neonates (≥ 35 weeks' gestation) with *CHD and acute perinatal stroke* confirmed by MRI born between 2007-2017 in Central-Hungary (Study Group). We compared their outcome to that of 56 subjects with *matching CHD without stroke* (Control-1) and 56 neonates with *matching perinatal stroke without CHD* (Control-2). The primary goal of the study was to investigate whether perinatal stroke in conjunction with CHD increases mortality and, if so, to identify the risk factors that might contribute to the suspected increase in mortality. The secondary aim was to

compare the long-term neurodevelopment outcome of the Study Group patients to infants in the control groups.

2.5 Perinatal stroke – review of the literature and proposed management guideline

In this section, the aim of the thesis is to provide a detailed review of the literature on perinatal stroke syndromes. We specifically focused on acute perinatal strokes, although we briefly also discuss studies on patients with presumed perinatal stroke. In this review, we summarize the epidemiology, imaging modalities, clinical presentation, and recommended management strategies as well as the available data on long-term neurodevelopmental outcome. In addition, we have also included our proposed management guideline on the diagnosis and management strategies of acute perinatal stroke.

3. Methods

We conducted a population-based study on neonates born between January 1, 2007, and December 31, 2017, in Central-Hungary including the capital, Budapest. In addition, children were also prospectively followed until December 31, 2018, for the purposes of this study.

MRI scans were performed at a single center, in the Department of Neuroradiology of Medical Imaging Centre, Semmelweis University. We reviewed the imaging studies of 1400 term and moderate/late preterm (≥ 32 weeks gestation) neonates who had a head MRI performed prior to 28 days of life and identified 225 subjects with MR-evidence of perinatal stroke.

We included all three types of acute perinatal stroke. In addition, periventricular injuries of fetuses and near-term infants were also regarded as stroke [81]. Finally, as Apgar scores of less than 7 at 5 minutes are associated with PAIS [58], we included cases with mild to moderate birth asphyxia if stroke without border-zone injury (watershed infarction) was described on MRI.

Due to the lack of diagnostic accuracy of head ultrasound for all forms of neonatal stroke, patients were only included if they also had a brain MRI confirming the diagnosis of perinatal stroke.

We obtained Institutional Review Board 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.

3.1 Patient selection in the study entitled “Perinatal stroke – from symptoms to follow-up”

This was a retrospective *and* prospective (i.e., longitudinal) analysis including 18 newborns (17 term and one near-term neonate) with acute MR-confirmed perinatal stroke. Inclusion criteria were: (1) term or near-term (≥ 36 weeks' gestation) neonates up to 28 days of age (2) and a brain MRI confirming the diagnosis of perinatal stroke

without any other concurrent abnormality. We excluded neonates with kernicterus, encephalitis, border-zone injury or periventricular leukomalacia on MRI, moderate-to-severe birth asphyxia, tumor, non-accidental brain injury, mitochondrial disorders, and congenital syndromes with known adverse outcome [2].

3.2 Patient selection in the study entitled “The role of brain territorial involvement and infection/inflammation in the long-term outcome of neonates with PAIS: a population-based cohort study”

As described in the article referenced [90], this was a population-based study designed to capture all term neonates with PAIS born between January 1 and December 31, 2017, and cared for in the Central-Hungarian region, including the capital, Budapest. We reviewed the imaging studies of 1400 neonates who had a head MRI performed prior to 28 days of life and identified 102 term neonates (≥ 37 weeks) with evidence of a focal cerebral arterial ischemic event. Patient selection is shown in [Figure 1](#). Inclusion criteria were: (1) term neonates up to 28 days of age (2) and a brain MRI confirming the diagnosis of PAIS without any other concurrent abnormality. We included neonates with CHD except those with single-ventricle physiology since CHD is a major risk factor for stroke [3]. 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 [2].

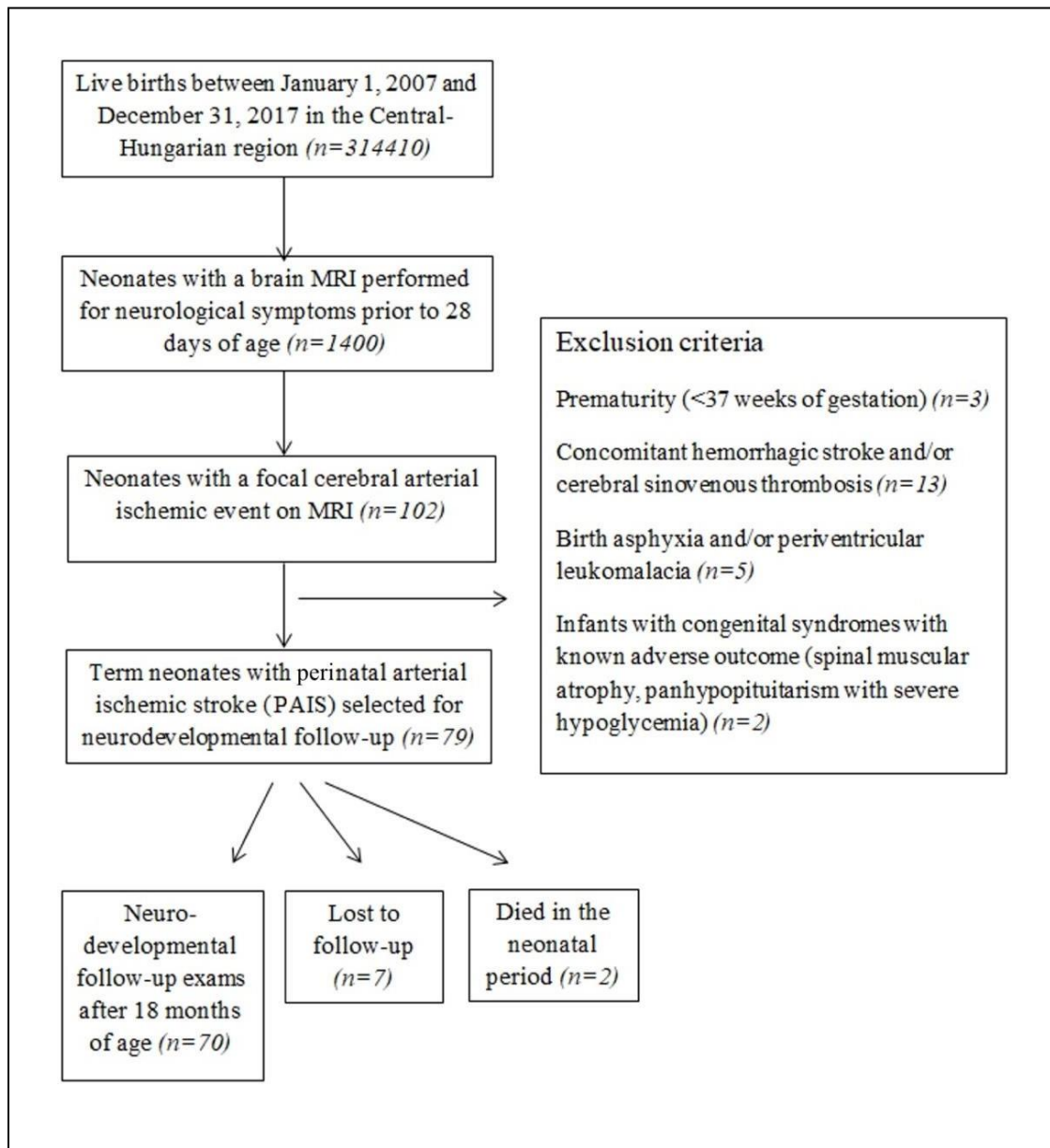


Figure 1. Process of patient selection, inclusion and exclusion criteria in the study entitled “The role of brain territorial involvement and infection/inflammation in the long-term outcome of neonates with perinatal arterial ischemic stroke: a population-based cohort study”

3.3 Patient enrollment in the study “Long-term neurodevelopmental outcome of term neonates with perinatal hemorrhagic stroke: a population-based study”

We performed a population-based study designed to capture all term and near-term neonates (≥ 36 weeks) with perinatal hemorrhagic stroke born between January 1, 2007 and December 31, 2017 in the Central-Hungarian region, including the capital, Budapest [81]. We reviewed the imaging studies of 1400 neonates who had a head MRI performed prior to 28 days of age and identified 96 infants with evidence of a focal hemorrhagic event. Patient selection is shown in [Figure 2](#). Inclusion criteria were: (1) neonates born at ≥ 36 weeks up to 28 days of age (2) and a brain MRI confirming the diagnosis of PHS without any other concurrent abnormality.

As did others [2], we also excluded neonates with encephalitis, tumor, non-accidental brain injury and birth asphyxia from the study. We also excluded neonates with exclusively intraventricular, subarachnoidal, subdural or epidural hemorrhage without intraparenchymal bleeding as well as neonates with periventricular hemorrhagic leukomalacia. Infants with congenital syndromes with known adverse neurodevelopmental outcome or other significant brain lesions were not addressed in this study.

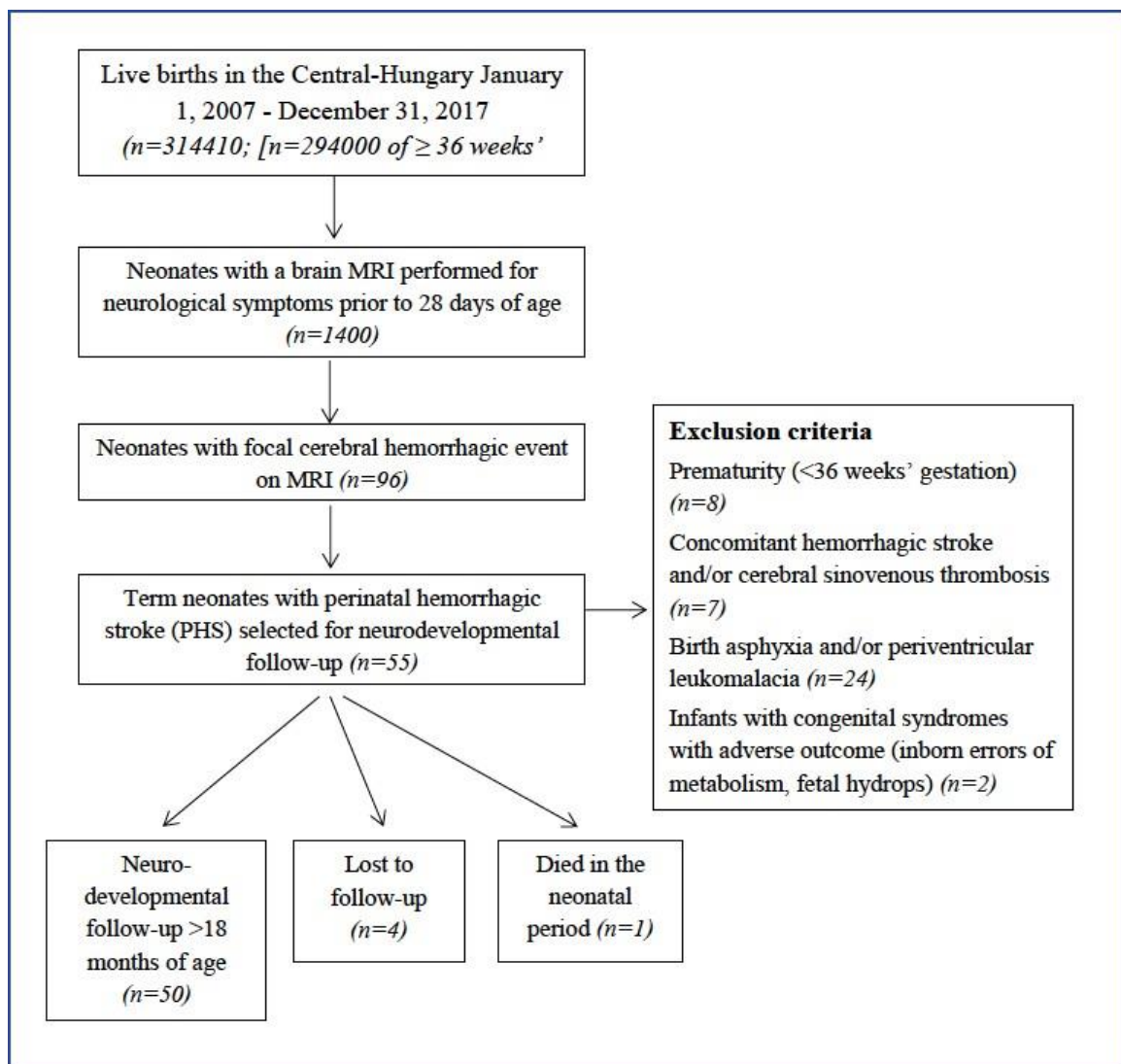


Figure 2. Course of patient selection, inclusion, and exclusion.

3.4 Patient enrollment in the study “Mortality and long-term outcome of neonates with congenital heart disease *and* acute perinatal stroke: a population-based case- control study”

We designed a population-based case-control study of term (37-41 weeks' gestation) and near- term (≥ 35 weeks' gestation) neonates with CHD *and* perinatal stroke (Study Group) born between the 1st of January 2007 and the 31st of December 2017 in Central-Hungary. Cardiac interventions were performed in a single center, in the Gottsegen Cardiology Intensive Care Unit, Budapest, Hungary. Neonates with acute

PAIS and acute PHS confirmed by MRI were included in the Study and Control-2 Groups. To decrease patient heterogeneity, we excluded neonates with CSVT. We included (1) term and near-term neonates up to 28 days of postnatal age,(2) brain MRI confirming the diagnosis of perinatal stroke without any other concurrent brain abnormality and (3) the presence of CHD. Neonates with CHD matching the cardiac diagnosis of the study infants but without perinatal stroke (Control-1) and subjects with perinatal stroke but without CHD (Control-2) were used as controls. Controls were recruited in a 1:2 ratio. Patient selection is shown in Figure 3A,B,C. Exclusion criteria included kernicterus, encephalitis, mitochondrial disorders, tumor, and non-accidental brain injury [24]. Neonates with presumed perinatal stroke, border-zone injuries secondary to asphyxia, epidural, subdural or intraventricular hemorrhage (without parenchymal injury) as well as bilateral preterm brain injury were also not included [24]. Patients with isolated ductus arteriosus with or without a patent foramen ovale were also excluded from the Study and Control-1 Groups.

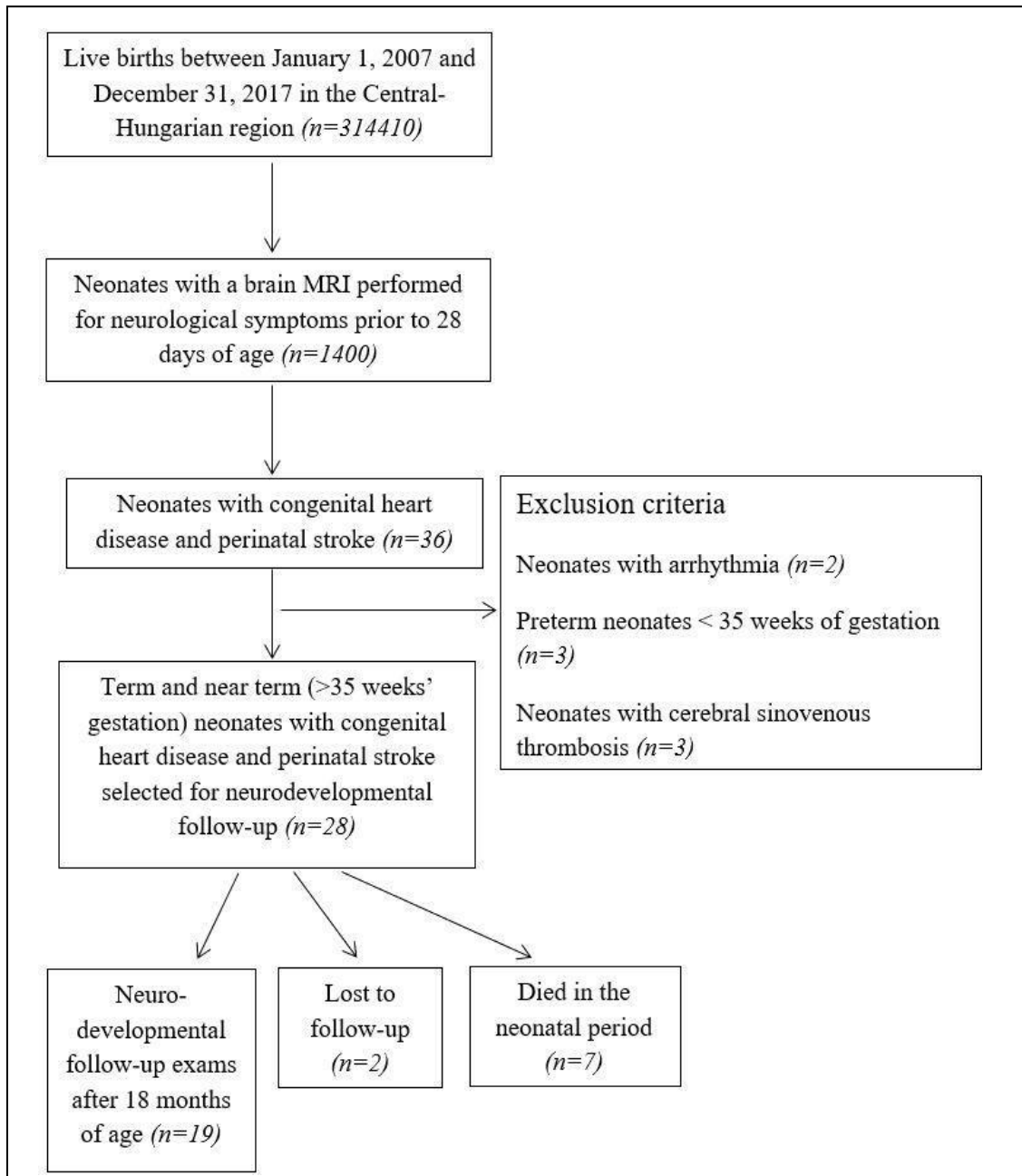


Figure 3A Patient selection and inclusion and exclusion criteria for neonates with congenital heart disease *and* acute perinatal stroke (Study Group).

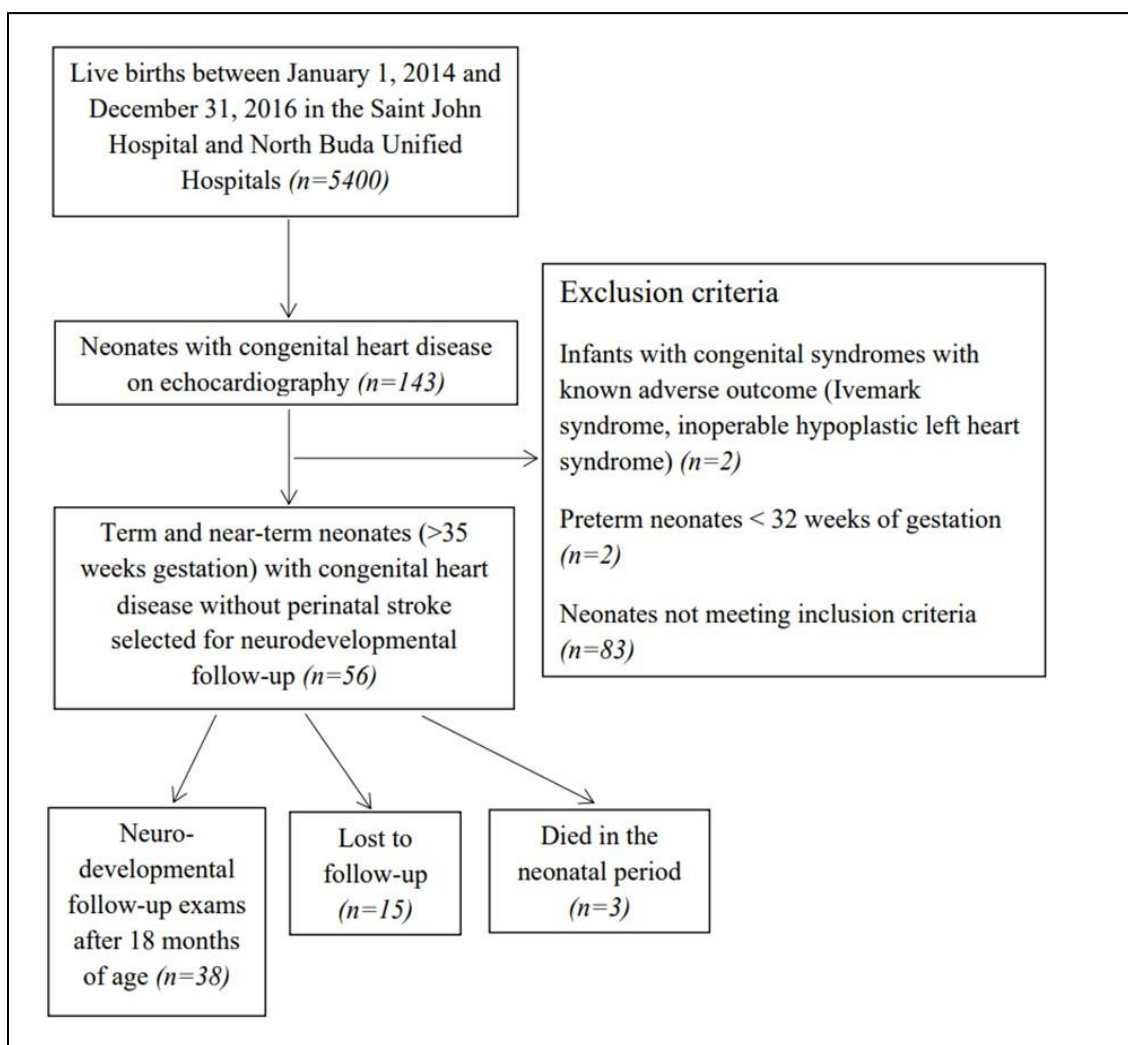


Figure 3B Patient selection and inclusion and exclusion criteria for neonates with congenital heart disease *without* acute perinatal stroke (Control-1 Group).

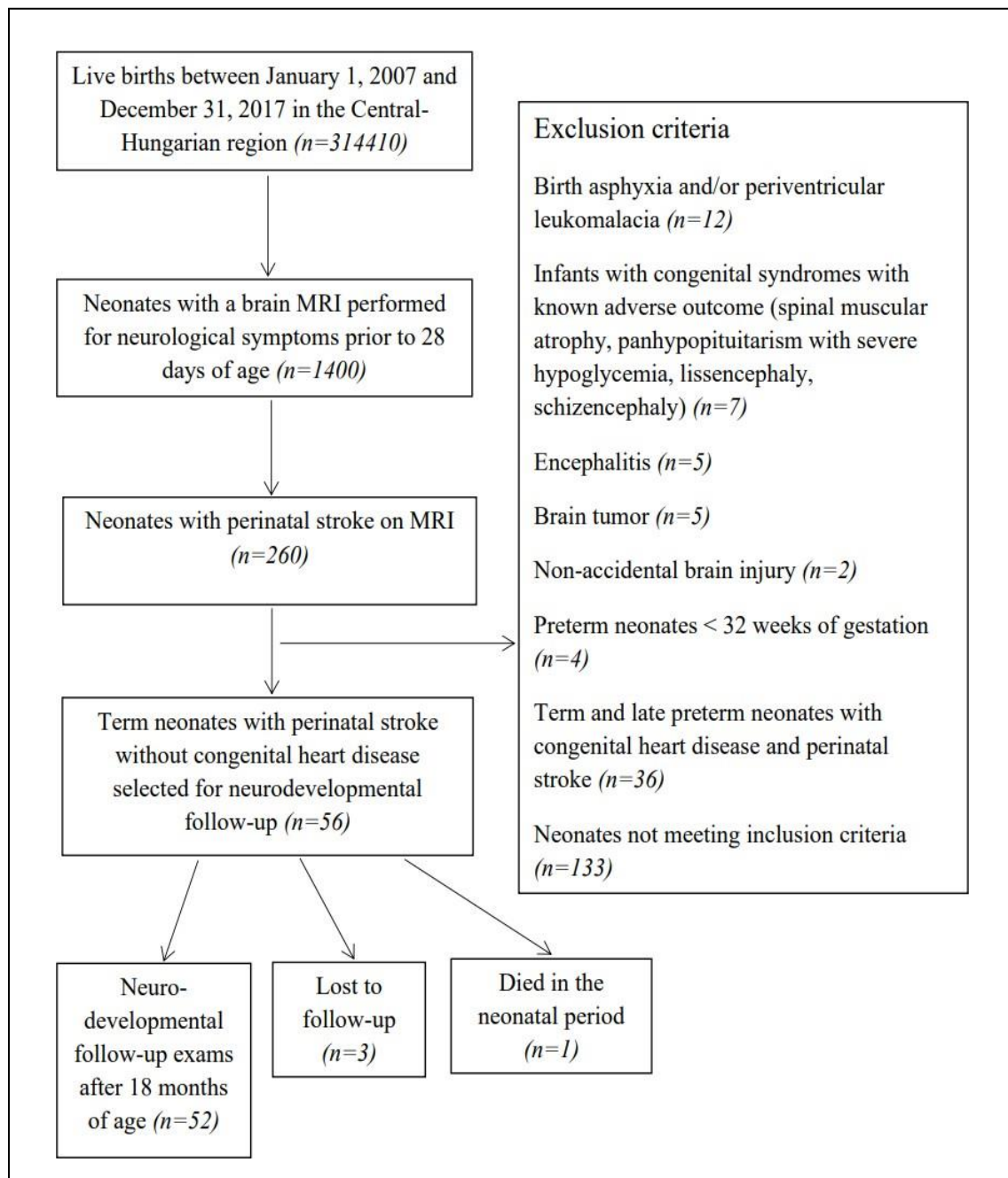


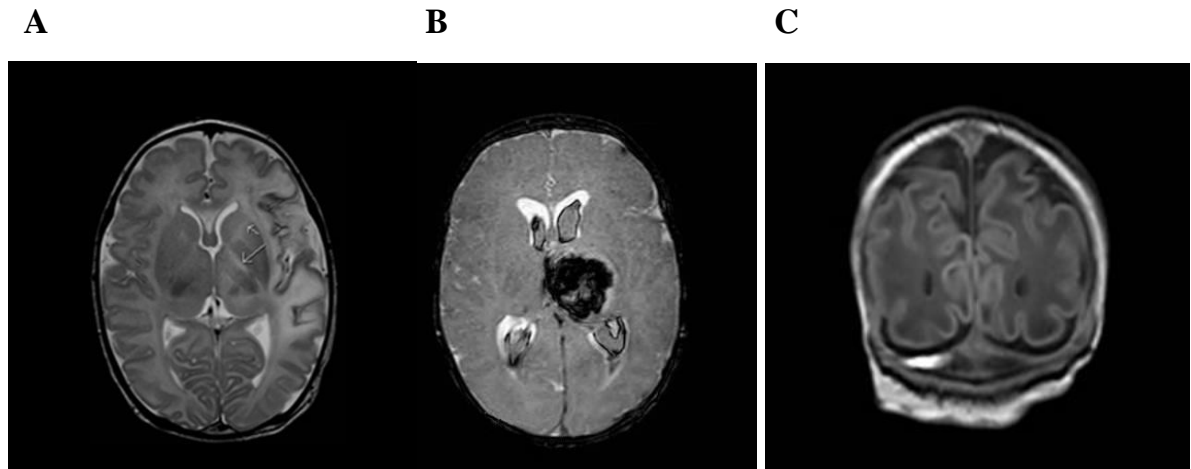
Figure 3C Patient selection and inclusion and exclusion criteria for neonates with acute perinatal stroke without congenital heart disease and/or arrhythmia (Control-2 Group).

3.5 MRI

Brain imaging was carried out on 3T Philips Achieva and 3T Philips Ingenia MR scanners (Philips Medical Systems, Best, The Netherlands) at the Department of Neuroradiology of Medical Imaging Centre, Semmelweis University, Budapest, Hungary. The scanning protocol included diffusion-weighted imaging, apparent diffusion coefficient map, conventional T1- and T2-weighted imaging, T2*- and susceptibility-weighted imaging. In selected cases, MR angiography and/or MR spectroscopy was added, as appropriate. Radiologists trained 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 [2, 13] in PAIS. Each stroke was assigned to one of the most predominant arterial territories. Infarction of the entire main MCA including the basal ganglia and corticospinal tract was attributed to the occlusion of the proximal segment of the MCA. A hemispheric lesion anterior to the central sulcus was assigned to the anterior branch of the MCA (Figure 4A), while the hemispheric lesion posterior to the central sulcus was assigned to the posterior branch of the MCA. The full central sulcus was attributed to being supplied by the middle MCA branch. Occlusion of the anterior cerebral artery (ACA), the posterior cerebral artery (PCA) or the perforator branch arteries supplying the deep gray matter (thalamus and/or basal ganglia) was also noted. Previous reports have described an association between involvement of the corticospinal tract (CST) and adverse neurodevelopmental outcome including CP [14,17,18]. Therefore, we also investigated the involvement of the CST seen as “pre-Wallerian degeneration” on diffusion-weighted imaging at the level of the PLIC and cerebral peduncles among neonates with arterial ischemic stroke. Multiple lesions were registered if more than one branch of the MCA were involved. Strokes of <3 mm in size at the largest diameter were not included in this study.

Among neonates with hemorrhagic stroke, classification was performed according to the affected brain compartment [81]. However, exclusively subarachnoid and other extra-axial hemorrhage without intraparenchymal hemorrhage is not included [81]. We have also noted the involvement of the thalamus and/or basal ganglia because previous reports have described an association between involvement of the thalamus and/or basal ganglia and adverse neurodevelopmental outcome (Figure 4B).

Figure 4.



A, Left anterior branch of the middle cerebral artery (MCA) PAIS with the involvement of the anterior and posterior branches (arrows) of the internal capsule on T2-weighted sagittal MRI.

B, Left PHS of the thalamus and basal ganglia on gradient-echo MR imaging.

C, Right transverse sinus CSVT on gradient-echo MR imaging.

As an additional approach, we measured the size of the PHS by drawing a region of interest around the area judged to have abnormally high signal intensity on T2*-weighted image using the 3D image analysis package of the MRICron program [82]. Lesion volumes were expressed as total volume (cm^3) as well as a percentage of supratentorial brain volume after excluding ventricular volume. The ratio of PHS to the supratentorial brain volume was further subcategorized as small (<5%), moderate (5%-10%) or large (>10%) based on the relative volume of the stroke.

3.6 Clinical data

Clinical information was collected from chart reviews. Gestational age, birthweight, sex, modes of delivery, evidence of fetal distress during labor, 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. Risk factors during pregnancy, such as gestational diabetes (GDM), preeclampsia, placental abruption and premature rupture of membranes (PROM) for more than 18 hours were collected retrospectively. We also analyzed neonates with possible birth injury (vacuum extraction, prolonged second stage labor and shoulder dystocia) as these clinical factors might place the neonate at risk for intracranial vessel injury, although this notion has been disputed.[24]

We also collected data on electroencephalogram (EEG) studies. The EEG signals were classified as seizure, abnormal background activity (including low voltage, flat trace, burst suppression, trace alternant, asynchrony or asymmetry) or normal [19]. Echocardiography was used as indicated to confirm/rule out congenital heart disease.

Although the utility of testing for coagulopathies remains controversial in patients with perinatal stroke [83], 69 patients in the present study had been investigated for genetic predisposition of thrombophilia (increased lipoprotein (a), factor V G1691A mutation, prothrombin G20210A variant, methylenetetrahydrofolate reductase (MTHFR) T677T genotype, antithrombin-III, protein-C and protein-S deficiency) as well as for acquired thrombophilia (antiphospholipid antibodies and Lupus anticoagulant). In addition, the presence of coagulation abnormalities in other conditions such as CSVT may also increase the risk of perinatal stroke.[83] Additionally, bleeding diathesis tests were performed in 15 neonates with PHS. Baseline bleeding diathesis tests included a full blood count, prothrombin time, activated partial thromboplastin time and thrombin time. If any one of these tests suggested the potential presence of an underlying coagulopathy, additional studies were performed. These included tests for neonatal alloimmune thrombocytopenia, platelet aggregation, von Willebrand factor disease/antigen, alfa2-plasmin inhibitor deficiency, as well as deficiency of the

following coagulation factors: fibrinogen, factor II/ V/ VII/ VIII/ IX/ X/ XI/ XII or XIII.

3.7 Neurodevelopmental outcome

In all patients, neurodevelopmental outcome was assessed during follow-up visits between the ages of 1.5 to 10 years. For the purposes of this study, we also prospectively performed a systematic neurodevelopmental follow-up assessment in children that were diagnosed with perinatal stroke and were available for follow-up in 2018. Normal outcome was defined as symptom-free survival. Adverse outcome was noted if 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. Neurodevelopmental outcome until up to three years of age was assessed using the Bayley Scales of Infant Development, Second Edition (BSID-II) mental and psychomotor developmental indices [84] or by the revised Brunet-Lézine scale (global developmental quotient score and sub-scores) [85]. Beyond three years of age, the Stanford-Binet Intelligence Scales (5th edition) was used to assess cognitive development [86]. We used data from after 18 months until 12 years of age. CP was diagnosed by using the criteria of the European Cerebral Palsy Surveillance Network [87]. Behavioral problems only after three years of age were considered and clinical psychologists or pediatric psychiatrists involved in the patients' care established the diagnosis. Epilepsy after the neonatal period was defined as more than two seizure episodes diagnosed on EEG and treated with standard anticonvulsive therapy and was classified according to the International League Against Epilepsy Commission on Classification and Terminology [88]. Visual field defects including hemianopia and quadrantanopia were diagnosed by pediatric ophthalmologists. To assess visual-motor functioning, the Bender- Gestalt test was performed. Language delay was defined as a language score of <-1 standard deviation (SD) on BSID-II or on the revised Brunet-Lézine scale, or as a diagnosis of speech and/or language disorder. Hearing loss was defined as a need for hearing aid or reduced electrophysiological activity on auditory tests including otoacoustic emissions and auditory evoked potentials.

3.8 Statistical analysis

Descriptive statistics were expressed as absolute numbers and percentages in the population studied. Mean and SD or median and interquartile range (IQR) were determined as appropriate. Neonatal clinical characteristics were compared between normal and adverse outcome groups using Fisher's exact test for categorical variables and the Mann–Whitney U-test for continuous variables. 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 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 previous research. Multicollinearity between variables was calculated with Spearman's rank correlation coefficient (ρ) prior to entering into the model. Models were checked according to the Hosmer and Lemeshow "goodness of fit" tests [89]. Where the omnibus chi-square test was significant, Wald tests and odds ratios (OR) with 95% confidence intervals (CI) were calculated for each clinical variable.

All statistical tests were two-sided, where p values of <0.05 were considered to indicate statistical significance. Statistical analyses were performed using IBM SPSS Statistics (Version 25, IBM Corp. Armonk, NY, USA).

4. Results

4.1 Perinatal stroke – from symptoms to follow-up

In this longitudinal study all patients were born in good condition with normal Apgar scores (9 ± 1). Cesarean section was performed in 4 cases. The diagnosis of perinatal stroke was confirmed by brain MRI performed on clinical suspicion of stroke in all except for three cases. In two patients, the diagnosis was based on fetal ultrasound findings, while one asymptomatic patient was diagnosed postnatally on routine ultrasound screening. Neurological symptoms presented in most patients during the first two postnatal days. In agreement with the data described in the literature, patients most frequently presented with seizures (77%). Seizure involved the hands and the face more often than the legs. Other neurological symptoms (irritability, apnea or hypotonia) were also frequently seen (39%). Only 17% of the patients presented with respiratory distress. Etiology confirmed in 14 of the 18 patients included thrombophilia (4/18), infection (4/18), vascular malformation (2/18), mild asphyxia (2/18) and pre-eclampsia (2/18). Middle cerebral artery was involved in 50% of the cases while the anterior cerebral artery was affected in 33%. The stroke occurred in the left hemisphere in 44% of the patients, the right side was affected in 39% and the stroke was bilateral in 17% of the cases.

Provision of early childhood developmental support was associated with average or above average gross and fine motor development, and cognitive outcome. Six patients were under 3 years of age. The results of the Brunet-Lézine developmental quotient (DQ) were > 110 (above average) in two of them, while 3 patients had average (90-110) scores. Not unexpectedly, severely impaired development was observed in the cases of in-utero stroke (one of them with a DQ of 48). The other patient with prenatally acquired stroke had to have an endoscopic fenestration of a residual cyst. Seven patients were more than 3 years' old: one patient had an intelligence quotient (IQ) of >120 (well above average), two had an IQ between 110-119 (above average) and four patients had normal (90-109) IQ.

4.2 Role of brain territorial involvement and infection/ inflammation in the long- term outcome of neonates with PAIS: a population-based cohort study

4.2.1 Patient characteristics and general findings

As described earlier [90], 79 neonates diagnosed with acute PAIS were identified in Central Hungary over the 11-year study period, resulting in 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 2](#).

Table 2. Clinical characteristics, maternal and neonatal risk factors and presenting symptoms of perinatal 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.

| | Patients with PAIS (n=79) |
|---|----------------------------------|
| Gestational age [weeks], <i>mean (SD)</i> | 38.8 (1.4) |
| Birthweight [g], <i>mean (SD)</i> | 3312 (592) |
| Normal birthweight, <i>n (%)</i> | 61 (77) |
| Large birthweight‡, <i>n (%)</i> | 10 (13) |
| Small for gestational age□, <i>n (%)</i> | 8 (10) |
| Male, <i>n (%)</i> | 50 (58) |
| Apgar score at 1 min, <i>mean (SD)</i> | 7 (3) |
| Apgar score at 5 min, <i>mean (SD)</i> | 9 (2) |
| Gestational diabetes mellitus, <i>n (%)</i> | 8 (11) |
| Preeclampsia, <i>n (%)</i> | 13 (16) |
| Placental abruption, <i>n (%)</i> | 17 (22) |
| C-section, <i>n (%)</i> | 42 (53) |
| 5 minute Apgar score <7, <i>n (%)</i> | 9 (11) |
| Possible birth trauma, <i>n (%)</i> | 6 (8) |

| | |
|--|---------------|
| Congenital heart disease, <i>n (%)</i> | 12 (15) |
| Cranial vasculopathy, <i>n (%)</i> | 9 (11) |
| Infection/Inflammation, <i>n (%)</i> | 15 (19) |
| Blood stream infection | 4 |
| Meningitis | 2 |
| Suspected sepsis | 9 |
| CRP in patients with infection, <i>median [IQR]</i> | 36 [13.5-5.5] |
| Clinical seizure, <i>n (%)</i> | 59 (75) |
| Postnatal day at first seizure, <i>median [IQR]</i> | 2 [1-3] |
| Hypoglycemia‡, <i>n (%)</i> | 20 (25) |
| Altered consciousness, <i>n (%)</i> | 10 (13) |
| Muscle tone abnormalities, <i>n (%)</i> | 13 (16) |
| Respiratory distress, <i>n (%)</i> | 29 (37) |
| Need for complex resuscitation and/or mechanical ventilation and/or cardiovascular support, <i>n (%)</i> | 7 (9) |

‡ Large birthweight was defined >4000g, □ Small for gestational age was defined <2500 g, †Hypoglycemia defined as blood glucose level <2.6 mmol/L

The most common presenting symptom of PAIS was seizure activity, occurring in 75% of patients, at a median age of 2 days [IQR 1-3]. Seizures were of focal onset in the majority of patients. Respiratory distress necessitating invasive or non-invasive ventilation was also frequent (37%). Interestingly, the rate of hypoglycemia was higher than in the general neonatal population, detected in 25% of the patients.

4.2.2 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 multiple strokes (n=33, 42%) were frequent. Detailed frequencies of stroke subtypes are shown in Table 3 and 4.

Table 3. MRI findings of territorial involvement and specific arteries affected by PAIS.

Percentages are shown in brackets.

| PAIS types and territorial involvement | Total n=79, (n%) |
|---|-------------------------|
| Main MCA, <i>n (%)</i> | 16 (20) |
| Anterior MCA, <i>n (%)</i> | 17 (22) |
| Middle MCA, <i>n (%)</i> | 11 (14) |
| Posterior MCA, <i>n (%)</i> | 15 (19) |
| Perforator stroke, <i>n (%)</i> | 9 (11) |
| Anterior cerebral artery, <i>n (%)</i> | 3 (4) |
| Posterior cerebral artery, <i>n (%)</i> | 9 (11) |
| PLIC +/- cerebral peduncles | 28 (35) |
| Multiple strokes, <i>n (%)</i> | 33 (42) |
| Right stroke, <i>n (%)</i> | 23 (29) |
| Left stroke, <i>n (%)</i> | 38 (48) |
| Bilateral strokes, <i>n (%)</i> | 18 (23) |

Abbreviations: MCA: middle cerebral artery, PLIC: posterior limb of internal capsule

Table 4. MRI findings on territorial involvement and the arteries affected by PAIS 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 (n=70). Percentages are shown in brackets.

| PAIS 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%) |
|-----------------------------|------------------|----------------------|---------------|---------------------------------|---------------------------------|--------------------------------|---|--------------------|------------------------------------|
| 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) |
| PLIC +/- cerebral peduncles | 28 (35) | 28 (35) | 17 (61) | 7 (25) | 8 (29) | 9 (32) | 13 (46) | 6 (21) | 24 (86) |
| Multiple strokes | 33 (42) | 32 (46) | 13 (41) | 5 (16) | 7 (22) | 11 (34) | 11 (34) | 7 (22) | 23 (72) |
| 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 (50) |
| Bilateral strokes | 18 (23) | 15 (21) | 5 (33) | 1 (7) | 4 (27) | 6 (40) | 7 (47) | 2 (14) | 11 (28) |

Abbreviations: MCA: middle cerebral artery, PCA: posterior cerebral artery, ACA: anterior cerebral artery, CP: cerebral palsy

Neonates with infection/inflammation did not have a 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 and higher rates of main MCA branch stroke than the other subgroups (54% vs. 44%, p=0.5 and 31% vs. 19%, p=0.5, respectively).

4.2.3 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 had died from complications of stroke and congenital heart disease in the neonatal period, three had visits between 12-18 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. In patients presenting with adverse neurodevelopmental outcomes, CP was the most frequent finding, documented in 20 patients (29%). Seventeen children (85% of patients with CP) had unilateral hemiparesis and 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%) and speech delay (23%) were common. Active epilepsy was documented at the time of assessment in 7 patients (10%). Neurodevelopmental outcome data are summarized in Table 5.

Table 5. Neurodevelopmental outcome data of patients with PAIS.

| | |
|---|---------|
| Long-term neurodevelopmental outcome data, <i>n (%)</i> | 70 (89) |
| Normal neurodevelopmental outcome, <i>n (%)</i> | 30 (43) |
| Cerebral palsy, <i>n (%)</i> | 20 (29) |
| GMFCS I. | 8 |
| GMFCS II. | 9 |
| GMFCS IV. | 2 |
| GMFCS V. | 1 |
| Fine motor impairment#, <i>n (%)</i> | 9 (13) |
| Mild | 4 |
| Moderate | 3 |
| Severe | 2 |
| Epilepsy, <i>n (%)</i> | 9 (13) |
| Seizure free without medication for >1 year | 2 |
| Active epilepsy | 7 |
| Cognitive deficit, <i>n (%)</i> | 12 (17) |
| Mild† | 5 |
| Moderate/ severe* | 7 |
| Behavioral problems, <i>n (%)</i> | 15 (21) |
| autism spectrum disorder | 1 |
| attention deficit hyperactivity | 2 |
| predominantly inattentive disorder | 7 |
| impulsive/aggressive behavior | 3 |
| special need in education and learning disorder | 1 |
| severe mental retardation | 1 |
| Language delay, <i>n (%)</i> | 16 (23) |
| Hearing loss, <i>n (%)</i> | 3 (4) |
| Visual field defect, <i>n (%)</i> | 2 (3) |
| Death in the neonatal period, <i>n (%)</i> | 2 (3) |

GMFCS: Gross Motor Function Classification System

#Fine motor impairment was classified according to the Santucci classification.

**Moderate-Severe impairment*: Stanford-Binet IQ <55; Revised Brunet-Lézine Developmental Quotient (DQ) <70; Bayley-II Mental Developmental Index Scores <70

†*Mild cognitive impairment*: Stanford-Binet IQ 79-55; Revised Brunet-Lézine DQ 70-84; Bayley-II Mental Developmental Index Scores 85-70

No cognitive impairment: Stanford-Binet IQ \geq 80; Revised Brunet-Lézine DQ \geq 85; Bayley-II Mental Developmental Index Scores of \geq 85

Eventhough 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 low (n= 7, 10%).

4.2.4 Risk factors associated with adverse neurodevelopmental outcome

Univariate analysis of stroke territory subtypes, clinical risk factors and their relation to neurodevelopmental outcome domains revealed several associations between MRI parameters and neurodevelopmental outcome domains (Table 6A, B).

Table 6A: Univariate associations between territorial involvement and specific arteries affected by PAIS and neurodevelopmental outcome domains expressed as odds ratios and 95% confidence intervals. Statistically significant associations are represented in **bold**.

| | Cerebral palsy OR [95% CI] | Fine motor impairment OR [95% CI] | Cognitive deficit OR [95% CI] | Behavioral problems OR [95% CI] | Visual/hearing/language problems OR [95% CI] | Epilepsy OR [95% CI] | Adverse outcome OR [95% CI] |
|--------------------------|----------------------------|-----------------------------------|-------------------------------|---------------------------------|--|---------------------------|-----------------------------|
| Main MCA | 44.6 [8.0-248.3] | NS | 5.4 [1.4-21.2] | 5.1 [1.4-18.6] | 7.7 [2.1-27.5] | 6.4 [1.4-28.7] | 7.3 [1.5-36.2] |
| Anterior MCA | NS | NS | NS | NS | NS | NS | NS |
| Medium MCA | NS | NS | NS | NS | NS | NS | NS |
| Posterior MCA | NS | NS | NS | 5.4 [1.4-21.2] | NS | NS | NS |
| Perforator | NS | NS | NS | NS | NS | NS | NS |
| PCA/ACA | NS | NS | NS | NS | NS | NS | NS |
| Multiple strokes | 3.0 [1.0- 9.1] | NS | NS | 4.5 [1.2-16.2] | NS | 5.04 [0.9-27.1] | 2.7 [1.0-7.4] |
| Left stroke | NS | NS | 0.008 [0.03-0.8] | 0.2 [0.017-0.7] | 0.3 [0.09-0.9] | NS | NS |
| Right stroke | NS | NS | NS | NS | NS | NS | NS |
| Bilateral strokes | NS | NS | NS | NS | NS | NS | NS |

Abbreviations: CI: confidence interval, OR: odds ratio, NS: non-significant, MCA: middle cerebral artery, PCA: posterior cerebral artery, ACA: anterior cerebral artery

Table 6B: Univariate associations between clinical risk factors and neurodevelopmental outcome domains in patients with PAIS expressed as odds ratios and 95% confidence intervals.

| | Cerebral palsy OR [95% CI] | Fine motor impairment OR [95% CI] | Cognitive deficit OR [95%CI] | Behavioral problems OR [95% CI] | Epilepsy OR [95% CI] | Language delay OR [95% CI] | Adverse outcome OR [95% CI] |
|---------------------------------|---|--|---|--|-------------------------------------|---|--|
| Congenital heart disease | NS | NS | NS | NS | NS | NS | NS |
| Possible birth trauma | NS | NS | NS | NS | NS | NS | NS |
| 5' Apgar <7 | NS | NS | NS | NS | NS | NS | NS |
| Arteriopathy | NS | NS | NS | NS | NS | NS | NS |
| Infection/ Inflammation | 3.9 [1.1-14.1] | 4.6 [1.0-21.1] | 7.3 [1.8-29.7] | NS | 8.3 [1.8-38.5] | 4.0 [1.1-14.9] | NS |
| Gestational diabetes | NS | NS | NS | NS | NS | NS | NS |
| Preeclampsia | NS | NS | NS | NS | NS | NS | NS |
| Placental abruption | NS | NS | NS | NS | NS | NS | NS |

Abbreviations: CI: confidence interval, OR: odds ratio, NS: non-significant

Main MCA stroke, multiple strokes and the involvement of the CST were associated with increased odds of overall adverse outcomes. Ninety-three% of patients with main branch MCA stroke had neurological impairment, compared to 29% to 67% of other PAIS subtypes. After separating patients with main MCA branch stroke, patients with CST involvement also had a high rate of adverse neurodevelopmental outcome (83%) compared to those without CST involvement (37%). Also, among the clinical risk factors, evidence for infection/inflammation conferred significantly higher odds for adverse outcome in several developmental domains.

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 7A](#)).

Table 7A: Results of multivariable logistic regression analysis represented by adjusted odds ratios and 95% confidence intervals predicting the likelihood of adverse outcomes in patients with PAIS and main MCA stroke and/or inflammation/infection. Statistically significant associations for aOR are shown in **bold**.

| | 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 |

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

Infants with main MCA stroke had 9.1 times higher odds (95% CI: 1.7-48.0) for overall adverse outcome, and main MCA branch infarction was associated with CP, cognitive and behavioral problems, epilepsy, and visual/hearing/language problems (Table 7A). 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 MCA branch stroke with multiple strokes (Table 7B) 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 7B: Results of multivariable logistic regression analysis represented by adjusted odds ratios and 95% confidence intervals predicting the likelihood of adverse outcomes in patients with PAIS and multiple strokes. Statistically significant associations are shown in **bold**.

| | 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 a OR [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

After excluding patients with main MCA branch stroke the involvement of PLIC with or without cerebral peduncles was associated with an increased risk for overall adverse outcome (OR: 8.1; 95% CI: 2.2-29.3) and for cerebral palsy/fine motor impairment (OR: 15.5; 95% CI: 3.6-67.5) (Table 7C).

Table 7C: 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 MCA stroke (n=55). Statistically significant associations are shown in **bold**.

| | Cerebral palsy | Cerebral palsy/Fine motor involvement | Adverse outcome |
|--|-----------------------|---------------------------------------|-----------------------|
| Descending corticospinal tract | 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: CI: confidence interval, aOR: adjusted odds ratio, NS: non-significant.

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 diagnosed in 17 (29%) subjects. 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).

4.3 Long-term neurodevelopmental outcome of term neonates with PHS: a population-based study

4.3.1 Patient characteristics and general findings

As described in the article [81], a total of 294,000 live births of ≥ 36 weeks' gestation were registered in the Central Hungarian Region over the 11-year study period. Acute PHS was diagnosed in 55 neonates, yielding a disease incidence of 1 per 5300 live births (prevalence of 1.87 with a 95% CI of [1.38, 2.36] per 10,000 live births for newborns of ≥ 36 weeks' gestation).

The most common presenting symptom of PHS was seizure activity, occurring in 61% of patients at a median age of 2 days [IQR 1,3]. The rate of respiratory distress was also relatively high, detected in 39% of the neonates.

Among the 55 infants with PHS, 36 (65%) had a maternal and/or a neonatal risk factor that might have contributed to the development of the PHS. Underlying etiologies included alloimmune thrombocytopenia ($n=3$), CHD ($n=5$) & cerebral vascular abnormalities ($n=6$) such as arteriovenous malformation ($n=4$), cavernoma ($n=1$) and venous angioma ($n=1$). In 10 patients (18%) more than one possible primary etiological factors were present suggesting that the etiology of PHS might have been multifactorial. Clinical characteristics of the study population are summarized in [Table 8](#).

Table 8: Clinical characteristics, maternal and neonatal risk factors and presenting symptoms of perinatal hemorrhagic stroke (PHS) 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 PHS, n=55 |
|---|--------------------------------|
| Gestational age [weeks], <i>mean (SD)</i> | 38.5 (1.6) |
| Birthweight [g], <i>mean (SD)</i> | 3317 (549) |
| Male, <i>n (%)</i> | 34 (62) |
| Apgar score 1 min, <i>mean (SD)</i> | 8 (2) |
| Apgar score 5 min, <i>mean (SD)</i> | 9 (1) |
| Possible birth trauma, <i>n (%)</i> | 9 (16) |
| Spontaneous vaginal birth, <i>n (%)</i> | 38 (69) |
| Elective C-section, <i>n (%)</i> | 11 (20) |
| Emergency C-section, <i>n (%)</i> | 6 (11) |
| Congenital heart disease, <i>n (%)</i> | 5 (9) |
| Cranial vasculopathy, <i>n (%)</i> | 6 (11) |
| Infection/Inflammation, <i>n (%)</i> | 11 (20) |
| CRP in patients with infection/inflammation, <i>median [IQR]</i> | 36 [13.5, 85.5] |
| Clinical seizure, <i>n (%)</i> | 34 (62) |
| Postnatal day at first seizure, <i>median [IQR]</i> | 2 [1,3] |
| Hypoglycemia‡, <i>n (%)</i> | 10 (18) |
| Irritability/lethargy, <i>n (%)</i> | 8 (15) |
| Muscle tone abnormalities, <i>n (%)</i> | 8 (15) |
| Respiratory distress, <i>n (%)</i> | 20 (36) |
| Complex resuscitation, <i>n (%)</i> | 4 (7) |

‡ Hypoglycemia was defined as blood glucose level <2.6 mmol/L, CRP: C-reactive protein.

4.3.2 Affected brain regions

Diagnostic brain MRI was performed at a median age of 5 [IQR 3, 7] days. Perinatal hemorrhagic stroke was typically unifocal (80%) and unilateral (89%). Stroke occurred in the frontal (24%), temporal (24%), parietal (18%), occipital lobes (18%), as well as in the basal ganglia and/or thalamus (22%). Eight patients (15%) developed hydrocephalus. Median stroke volume was 10.6 cm³ [IQR 2.2, 22.8] with a median stroke percentage of the supratentorial brain volume of 2.7% [IQR 0.7, 6.8]. Most of the strokes were subcategorized as small ($n=34$, 62%), with the minority as moderate ($n=15$, 27%) or large ($n=6$, 11%). Detailed frequencies of stroke subtypes are shown in Table 9.

Table 9: MRI findings of territorial involvement and specific arteries affected by perinatal hemorrhagic stroke (PHS) and neurodevelopmental 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. Percentages are shown in brackets.

| PHS types and outcomes Total <i>n</i> =55, (%) | Follow-up <i>n</i> =50 | CP <i>n</i>=8, (%) | Cognitive impairment <i>n</i> =7, <i>n</i> (%) | Behavioral problems <i>n</i> =12, (%) | Visual /hearing problems <i>n</i> =7, (%) | Language disorder <i>n</i> =9, (%) | Epilepsy <i>n</i> =9, (%) | VP shunt <i>n</i> =9, (<i>n</i> %) | Overall chronic developmental sequelae <i>n</i> =30, (%) |
|---|----------------------------------|-------------------------------|--|---|---|--|--|---|--|
| Frontal lobe <i>n</i> =13 (24) | 12 | 0 (0) | 0 (0) | 2 (15) | 1 (8) | 2 (15) | 0 (0) | 1 (8) | 5 (42) |
| Temporal lobe <i>n</i> =13 (24) | 12 | 4 (33) | 3 (25) | 4 (33) | 2 (17) | 3 (25) | 4 (33) | 3 (25) | 7 (58) |
| Parietal lobe <i>n</i> =10 (18) | 9 | 4 (44) | 5 (56) | 3 (33) | 2 (22) | 3 (33) | 2 (22) | 2 (22) | 6 (66) |
| Occipital lobe <i>n</i> =10 (18) | 9 | 1 (11) | 1 (11) | 3 (33) | 3 (33) | 1 (11) | 2 (22) | 2 (22) | 6 (66) |
| Thalamus +/- basal ganglia <i>n</i> =12 (21) | 12 | 3 (25) | 1 (8) | 3 (25) | 2 (17) | 1 (8) | 6 (50) | 3 (25) | 7 (58) |
| Cerebellum <i>n</i> =2 (4) | 2 | 0 (0) | 0 (0) | 1 (50) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (50) |
| Multiple territories <i>n</i> =11 (20) | 10 | 4 (40) | 3 (30) | 4 (40) | 3 (30) | 2 (20) | 6 (60) | 4 (40) | 8 (80) |
| Right stroke <i>n</i> =28 (51) | 24 | 4 (17) | 5 (21) | 6 (25) | 3 (13) | 5 (21) | 4 (17) | 5 (21) | 14 (58) |
| Left stroke <i>n</i> =21 (38) | 21 | 3 (14) | 2 (10) | 6 (29) | 2 (10) | 1 (5) | 5 (24) | 4 (19) | 9 (43) |
| Bilateral strokes <i>n</i> =6 (11) | 5 | 1 (20) | 0 (0) | 0 (0) | 2 (40) | 3 (60) | 2 (40) | 0 (0) | 4 (80) |
| Small strokes <i>n</i> =34 (62) | 32 | 3 (9) | 3 (9) | 6 (19) | 3 (9) | 5 (16) | 6 (19) | 4 (13) | 16 (50) |
| Moderate strokes <i>n</i> =15 (27) | 13 | 3 (23) | 2 (15) | 6 (46) | 3 (23) | 2 (15) | 3 (23) | 4 (31) | 8 (62) |
| Large strokes <i>n</i> =6 (11) | 5 | 2 (40) | 2 (40) | 0 (0) | 1 (20) | 2 (40) | 2 (40) | 1 (20) | 4 (80) |

Number of infants with completed follow-up for at least 18 months of age per stroke territory subtypes are presented in “Follow-up” column.

Small strokes: <5% of supratentorial brain volume,

Moderate strokes: 5-10% of supratentorial brain volume,

Large strokes: >10% of supratentorial brain volume.

CP: cerebral palsy. VP: ventriculoperitoneal

4.3.3 Neurodevelopmental outcome

Long-term neurodevelopmental outcome data were available in 50 (91%) of the 55 children with the last follow-up visit occurring at a median age of 60 [IQR 35, 88] months. Fifteen children were assessed by the Brunet-Lézine ($n=13$) or the Bayley-II scale ($n=2$) at a median age of 24 months [IQR 19, 34], while 30 children were evaluated by the Stanford-Binet Intelligence Scales at a median age of 74 months [IQR 60, 108]. Finally, five children were followed by a pediatric neurologist without using a formal neurodevelopmental assessment due to their severe neurodevelopmental delay. Of the five infants lost to follow-up, one died from complications of stroke *and* CHD in the neonatal period, two children with neurodevelopmental findings meeting population norms between 12-18 months of age were lost to further follow-up thereafter, and two patients were lost to follow-up after the neonatal period. All infants lost to follow-up had unilateral and unifocal territorial involvement on MRI not affecting the basal ganglia and/or thalami.

Twenty of the 50 infants (40%) with longitudinal follow-up had been developing according to the population norms. In individuals with chronic neurodevelopmental sequelae, the most common outcomes were behavioral problems (24%), epilepsy (22%) and language disorders (18%). As out of the eleven patients (22%) initially diagnosed with epilepsy, two children subsequently remained seizure free for more than a year without antiepileptic treatment, the rate of active epilepsy was 18% among the individuals in the end. Unexpectedly, CP was not as frequent finding as in neonates with PAIS [90]. Indeed, CP was only documented in 8 patients (16%), yet in those with CP, the condition was more severe as 75% of the patients (6 out of 8) presented with tetraparesis. Children developed visual field defect (8%) and hearing loss (6%) infrequently. Thirteen individuals (26%) were recorded with more than one type of developmental sequelae.

4.3.4 Risk factors associated with adverse neurodevelopmental outcome

None of the neonates with frontal lobe PHS developed later CP, cognitive impairment, or epilepsy during the follow-up period. The overall rate of impaired neurodevelopmental outcome was also less (42% vs 50-66% in other lobes).

Univariate analysis of stroke territory subtypes and their relation to neurodevelopmental outcome domains revealed several associations between brain territorial involvement and neurodevelopmental outcome domains. Among the clinical risk factors, the concomitant presence of CHD conferred significantly higher odds for cognitive impairment and clinical seizure on admission was associated with developing epilepsy beyond the neonatal period.

Multivariable logistic regression analysis revealed that parietal lobe PHS is a significant independent predictor of CP (OR: 6.7 95% CI: 1.1-41.4) and cognitive deficit (OR: 23.6 95% CI: 2.9-194.9) (Table 10A). Infants with basal ganglia and/or thalamus involvement had seven times higher odds (95% CI: 1.3-37.7) for epilepsy (Table 10B). Strokes involving multiple lobes were independent predictors of impairment in several neurodevelopmental domains including CP (OR: 6.7 95% CI: 1.0-40.5), epilepsy (OR: 10.8 95% CI: 1.8-64.3) and the need for ventriculoperitoneal (VP) shunt placement (OR: 5.7 95% CI: 1.0-30.7) (Table 10C).

Table 10: Results of multivariable logistic regression analysis represented by adjusted odds ratios and 95% confidence intervals predicting the likelihood of chronic neurodevelopmental sequelae in patients with parietal lobe stroke (**A**), thalamus and/or basal ganglia hemorrhage (**B**), and multiple strokes (**C**) while controlling for other clinically relevant factors. Statistically significant associations are shown in **bold**.

A

| | Cerebral palsy | Cognitive impairment |
|--|---------------------------|-----------------------------|
| Parietal NHS aOR [95% CI] | 6.7 [1.1-41.4] | 23.6 [2.9-194.9] |
| Emergency C-section, aOR [95% CI] | 0.5 [0.03-9.8] | 0.3 [0.009-9.0] |
| CHD aOR [95% CI] | 1.5 [0.09-25.8] | 6.8 [0.4-110.6] |
| Seizure aOR [95% CI] | 5.2 [0.5-54.5] | 1.5 [0.1-15.6] |

B

| | Epilepsy |
|--|-----------------------|
| Thalamus +/- basal ganglia aOR [95% CI] | 7.0 [1.3-37.7] |
| Emergency C-section aOR [95% CI] | 1.3 [0.09-18.7] |
| CHD aOR [95% CI] | 2.9 [0.2-49.1] |
| Seizure aOR [95% CI] | 8.8 [1.0-81.7] |

C

| | Cerebral palsy | Epilepsy | VP shunt placement |
|----------------------------|-----------------------|-------------------|---------------------------|
| Multiple strokes | 6.7 | 10.8 | 5.7 |
| aOR [95% CI] | [1.0-40.5] | [1.8-64.3] | [1.0-30.7] |
| Emergency C-section | 1.8 | 1.3 | |
| aOR [95% CI] | [0.1-26.7] | [0.09-19.7] | -* |
| CHD | 3.8 | 3.1 | -* |
| aOR [95% CI] | [0.2-61.5] | [0.2-50.9] | |
| Seizure | 4.7 | 8.3 | 0.5 |
| aOR [95% CI] | [0.5-45.2] | [0.9-79.8] | [0.1-2.7] |

Abbreviations: CI: confidence interval, aOR: adjusted odds ratio, NS: non-significant, CHD: congenital heart disease.

*Based on the findings of the univariate analysis, these variables were not entered into the multivariable analysis.

When a logistic regression model was fitted, there was no relationship between infarct size category and neurodevelopmental outcomes. This finding suggests that the location of the stroke is more important in predicting the neurodevelopmental outcome than the volume of the lesion itself.

Finally, among the clinical risk factors, clinical seizure on admission was associated with the risk of later developing epilepsy (OR: 8.8 95% CI: 1.0-81.7). Indeed, neonates with clinical seizure on admission developed epilepsy more frequently than those without clinical seizure on admission (30% vs 5%, $p=0.03$).

4.4 Mortality and long-term outcome of neonates with congenital heart disease *and* acute perinatal stroke: a population-based case-control study

4.4.1 Clinical characteristics

A total of 1400 MR images obtained prior to 28 days postnatal life were reviewed. Altogether 225 patients were diagnosed with acute perinatal stroke in Central-Hungary over the 11-year study period yielding a disease incidence of 1 per 1400 live births. Echocardiography confirmed CHD in 28 patients (Table 11), resulting in an incidence of neonates with CHD *and* perinatal stroke of 1 per 11000.

Table 11: Types of cardiac disease in all neonates with perinatal stroke and congenital heart disease.

| Types of cardiac disease in neonates with CHD and perinatal stroke | Frequency | Percent |
|--|-----------|------------|
| TGA | 11 | 39.3 |
| ASD | 5 | 17.9 |
| Supracardiac Total Anomalous Pulmonary Venous Return | 3 | 10.7 |
| VSD | 2 | 7.1 |
| Aorticopulmonary Fenestration, VSD | 1 | 3.6 |
| Critical Aortic Valve Stenosis | 1 | 3.6 |
| Critical Pulmonary Stenosis | 1 | 3.6 |
| Double Inlet Left Ventricle, ASD | 1 | 3.6 |
| Interrupted Aortic Arch, VSD, ASD | 1 | 3.6 |
| Pulmonary Atresia with Ventricular Septal Defect | 1 | 3.6 |
| Tetralogy of Fallot | 1 | 3.6 |
| Total | 28 | 100 |

ASD: atrial septum defect, CHD: congenital heart disease,

TGA: transposition of the great arteries, VSD: ventricular septum defect.

Of note is that two-thirds of the neonates with perinatal stroke and congenital heart disease were diagnosed with cyanotic CHD lending further support to the notion of an increased propensity to brain injury seen in children with cyanotic CHD.

Comparison of the baseline characteristics, stroke types and the frequencies of cardiac interventions/open-heart surgeries of the Study Group and of Control-1 and Control-2 Groups revealed several statistically significant differences (Table 12).

Table 12: Baseline clinical characteristics, stroke types and the frequencies of cardiac interventions/open-heart surgeries of patients in the Study Group and in the Control-1 (CHD without perinatal stroke) and Control-2 (perinatal stroke without heart disease) Groups. Number of cases with percentages in brackets is given for discrete data. For continuous variables, mean \pm standard deviations are shown. Fisher Exact tests were run for frequencies, while one-way ANOVA models were used to investigate comparison of group means for continuous variables.

| | Study Group (CHD and perinatal stroke) | Control-1 Group (CHD without perinatal stroke) | Control-2 Group (Perinatal stroke without CHD) | p-value |
|---------------------------------|--|--|--|----------------|
| Total number | 28 (100.0%) | 56 (100.0%) | 56 (100.0%) | NA |
| Gender | | | | |
| Female | 8 (28.6%) | 21 (37.5%) | 26 (46.4%) | 0.23 |
| Male | 20 (71.4%) | 35 (62.5%) | 30 (53.6%) | |
| Birthweight | 3166.2 (\pm 662.3) | 2956.6 (\pm 879.6) | 3287.9 (\pm 566.4) | 0.1 |
| Gestational age | 38.2 (\pm 1.5) | 37.5 (\pm 1.5) | 38.5 (\pm 1.3) | 0.003** |
| Apgar 1 | 7.6 (\pm 2.5) | 8.4 (\pm 1.7) | 7.3 (\pm 2.4) | 0.02* |
| Apgar 5 | 8.7 (\pm 1.8) | 9.3 (\pm 1.0) | 8.6 (\pm 1.9) | 0.04* |
| Mode of delivery | | | | |
| Vaginal | 17 (60.7%) | 6 (10.7%) | 29 (51.8%) | <0.001*** |
| Cesarean section | 11 (39.3%) | 50 (89.3%) | 27 (48.2%) | |
| Twins/Triplets | 2 (7.1%) | 10 (17.9%) | 0 (0%) | 0.001** |
| Arterial ischemic stroke | 16 (57%) | NA | 43 (54%) | 0.08 |
| Middle cerebral | 11 (69%) | NA | 32 (74%) | 0.7 |

| | | | | |
|--|----------|----------|----------|-------|
| artery | | | | |
| Anterior cerebral artery/ Posterior cerebral artery | 5 (31%) | NA | 11 (26%) | |
| Hemorrhagic stroke | 12 (43%) | NA | 13 (31%) | |
| Small stroke | 15 (54%) | NA | 30 (54%) | >0.99 |
| Large stroke | 13 (46%) | NA | 26 (46%) | |
| Cardiac intervention^o | | | | |
| Yes | 17 (61%) | 19 (38%) | NA | 0.03* |
| Occurrence of stroke prior to intervention | 9 (53%) | NA | NA | NA |
| Occurrence of stroke after intervention | 8 (47%) | NA | NA | |
| No | 11 (39%) | 37 (62%) | NA | |
| Open-heart surgery | | | | |
| Yes | 8 (29%) | 10 (14%) | NA | 0.3 |
| Occurrence of stroke prior to surgery | 1 (13%) | NA | NA | NA |
| Occurrence of stroke after surgery | 7 (87%) | NA | NA | |
| No | 20 (71%) | 46 (86%) | NA | |

Small stroke: Strokes of <3 cm size at the largest diameter

Large stroke: Strokes of ≥ 3 cm size at the largest diameter

^oCardiac interventions included open-heart surgery, Rashkind septostomy, balloon valvuloplasty and diagnostic cardiac catheterization.

NA: not applicable, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Neonates with CHD only (Control-1 Group) were born at a significantly younger gestational age than the neonates in the Study and Control-2 Groups ($p=0.003$). In addition, neonates in the Control-1 Group were born via C-section at a higher rate than infants in the Study and Control-2 Groups (89% vs. 39% and 48%, $p<0.001$). The occurrence of cardiac interventions including Rashkind septostomy or open-heart surgery in the neonatal period was significantly higher among subjects in the Study Group compared to the Control-1 Group (61% vs. 38%, $p=0.01$) (Table 12). There was no statistically significant difference in the frequency of stroke types between the Study and Control-2 Groups (Table 12).

4.4.2 Long-term outcome of neonates with heart disease *and/or* stroke

After excluding patients who died in the neonatal period, long-term neurodevelopmental outcome data were available in 19 (90%) of the 21 surviving Study Group patients (CHD *and* perinatal stroke), 38 (72%) of the 53 surviving infants in the Control-1 Group (CHD without perinatal stroke) and 52 (95%) of the 55 surviving subjects in the Control-2 Group (perinatal stroke without CHD). The last follow-up visit took place at a median age of 61 months [range 18-144 months]. Neonates who were lost to follow-up did not differ regarding the type of stroke or CHD from the subjects completing neurodevelopmental follow-up.

Compared to the control groups, mortality was highest in neonates with CHD *and* perinatal stroke (25%, $p=0.001$). Of note is that mortality was the lowest in patients with stroke without CHD (Control-2 Group, 2%). Data on neurodevelopmental outcome after 18 months of age revealed that normal outcome was most prevalent in the surviving patients of the Control-1 Group (CHD only, 74%) compared to the patients in the Study (47%) or Control-2 Groups (48%, $p=0.03$). Accordingly, abnormal neurodevelopmental outcome was higher in the Study (53%) and Control-2 Groups (52%) compared to the subjects in the Control-1 Group (26%, $p=0.03$) (Table 13). The rate of cerebral palsy was highest among patients with perinatal stroke only (Control-2), while the rate of cognitive impairment and epilepsy was highest in the Study Group. Altogether, six children (32%) in the Study Group experienced at least 1 seizure after the neonatal

period. However, after excluding one patient with febrile seizures and another one who was initially diagnosed with epilepsy but was later discontinued to take antiepileptic medication and remained seizure-free for more than one year without treatment, the rate of active epilepsy in the Study Group was 21% at the time of the last neurodevelopmental follow-up (Table 13).

Table 13: Mortality (A) and neurodevelopmental outcome after 18 months of follow-up (B) in patients with CHD and perinatal stroke (Study Group) compared to patients with CHD without perinatal stroke (Control-1 Group) and to patients with perinatal stroke but without CHD (Control-2 Group). Fisher's exact tests were run to assess the statistical comparison between each investigated outcome of the Study and the Control Groups.

A

| Overall neonates enrolled in the study | Study Group (n=28) | Control-1 Group (n=56) | Control-2 Group (n=56) | p-value |
|--|--------------------|------------------------|------------------------|----------|
| Death in neonatal period | 7 (25%) | 3 (5%) | 1 (2%) | 0.001 ** |
| Survival | 21 (75%) | 53 (95%) | 55 (98%) | |

**p-value <0.01

B

| Neonates with long-term follow-up (≥18 months) | Study Group (n=19) | Control-1 Group (n=38) | Control-2 Group (n=52) | p-value |
|--|--------------------|------------------------|------------------------|---------|
| Normal | 9 (47%) | 28 (74%) | 25 (48%) | 0.03* |
| Abnormal | 10 (53%) | 10 (26%) | 27 (52%) | |
| Cerebral palsy | 3 (16%) | 4 (11%) | 15 (29%) | 0.08 |
| Cognitive impairment | 4 (21%) | 5 (13%) | 9 (17%) | 0.7 |
| Epilepsy | 4 (21%) | 2 (5%) | 8 (15%) | 0.1 |
| Behavioral | 2 (11%) | 5 (13%) | 11 (21%) | 0.5 |

| | | | | |
|----------------------------|---------|---------|---------|-----|
| problems | | | | |
| Language disorder | 3 (16%) | 5 (13%) | 8 (15%) | 0.9 |
| Visual field defect | 0 (0%) | 2 (5%) | 2 (4%) | 0.8 |
| Hearing loss | 0 (0%) | 3 (8%) | 2 (4%) | 0.6 |

* p -value <0.05

Finally, in the Study group, we could not identify any difference among the risk factors in subjects who died compared to those who survived, and among those with normal outcome compared to those with adverse outcome (data not shown).

4.4.3. Risk factors associated with mortality

Multivariable regression analysis was fitted to investigate the risk of mortality in the Study and Control-1 and Control-2 Groups, while adjusting for clinically relevant parameters, including gestational age at birth, Apgar score at 1 min, vaginal birth, twin pregnancy and cardiac interventions. First, Control-1 Group was selected as a reference variable (Table 14) and then we repeated the analysis using Control-2 Group as a reference variable.

Table 14: Results of logistic regression analysis represented by *p* values, adjusted odds ratios and 95% confidence intervals predicting the likelihood of death based on clinically relevant risk factors in patients with congenital heart disease and acute perinatal stroke (Study Group) and acute perinatal stroke without congenital heart disease (Control-2). Neonates with congenital heart disease without stroke (Control-1) were selected as a reference variable.

| | Adjusted | |
|---------------------------------|-----------------|----------------|
| | OR (95% CI) | <i>P</i> value |
| Control-1 Group | NA | 0.1 |
| Control-2 Group | 3.1 (0.2-42.3) | 0.4 |
| Study Group | 6.5 (1.1-39.4) | 0.04* |
| Vaginal birth | 0.3 (0.06-1.6) | 0.2 |
| Gestational age at birth | 1.4 (0.8-2.5) | 0.3 |
| Twin pregnancy | 1.6 (0.08-33.1) | 0.8 |
| Apgar 1 | 1.1 (0.7-1.8) | 0.7 |
| Cardiac interventions | 8.1 (0.9-73.5) | 0.06 |

OR: odds ratio, CI: confidence interval, NA: not available, **p* < 0.05

Of the predictor variables, patients in the Study Group had 6.5 times higher odds for mortality compared to patients in the Control-1 Group (95% CI: 1.1-39.4) while adjusting for all the other relevant potentially confounding clinical parameters.

Then we used the Control-2 Group as the reference variable in the multivariable logistic regression model adjusted for the clinically relevant parameters. In this model, the risk of mortality was not increased in the Study Group compared to the Control-2 Group. Additionally, no other parameters were found to be significantly different in the logistic regression models (data not shown).

4.5 Neonates with CSVT and neonates with fetal stroke

Twenty-eight neonates were diagnosed with CSVT over the 11-year study period in Central- Hungary, resulting in an average annual incidence of CSVT of one in 11000 live births. [91] As a rule of a thumb, CSVT had poor prognosis, as 18% of the patients died in the neonatal period, and among those, who survived and whom were followed-up for at least 18 months of age, 70% of the patients had adverse long-term neurodevelopmental outcome. [91] Three-quarters of the neonates with CSVT had thrombosis in multiple cerebral sinuses. [91] The most commonly affected sinus was the transverse sinus (82%) (Fig 4C).

Stroke developed in fetal life in 20 neonates (9% of all neonates diagnosed with acute perinatal stroke). None of the died in the neonatal period, however long-term neurodevelopmental outcome was adverse in 67% of the neonates with fetal stroke. [91] Three patients with intrauterine stroke were diagnosed prior to delivery during routine ultrasound scan, while 17 neonates were diagnosed after delivery based on their clinical symptoms. In these cases, the MR imaging confirmed the development of the stroke antenatally. [91]

4.6 Investigation and management guideline

Based on recommendations published in a recent review [92], we have designed a guideline [93] to aid the clinician in the process of establishing the diagnosis and management plan in neonates with perinatal stroke. Since this is a guideline and not a protocol, decision making during the process may be modified to best fit the characteristics and needs of the individual patient.

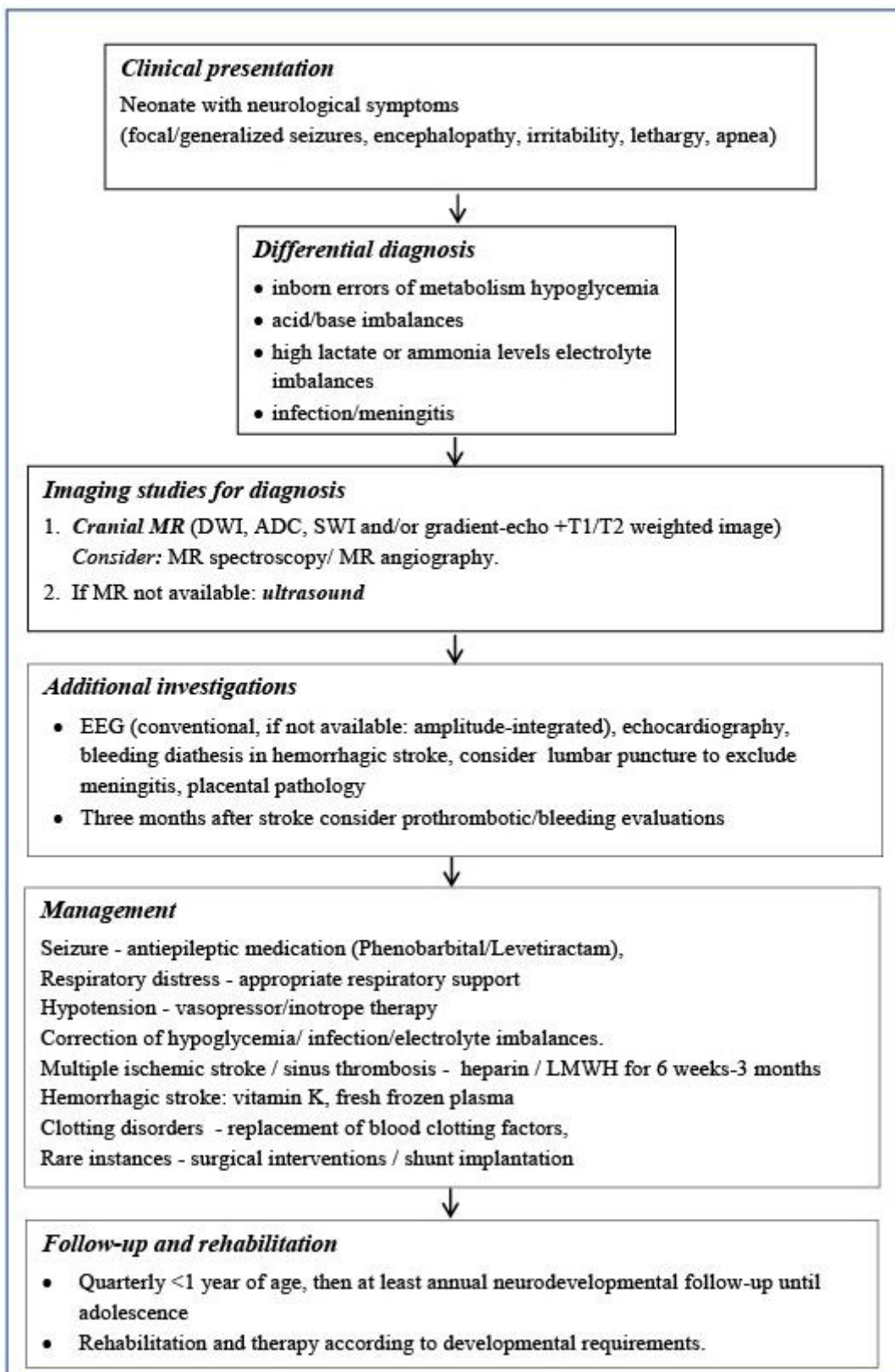


Figure 5. Guideline for the diagnosis and care of neonates with acute perinatal stroke

5. Discussion

First, we conducted a longitudinal case-series at the 3rd level Neonatal Intensive Care Unit of the Szent János Hospital, Hungary that aimed to analyze the presentation, imaging methods, etiology, and clinical context of perinatal stroke of term neonates with perinatal stroke born between 2006 and 2017. All patients had a follow-up examination at 2 years of age or later. We found that neonates with perinatal stroke are generally born at good condition with normal Apgar scores, and the symptoms of perinatal stroke occur within the first two days of postnatal life. The most common symptom similarly to described in the literature, is seizure, nevertheless, diffuse neurological symptoms such as irritability/apnea or hypotonia, as well as respiratory distress are also not infrequent. Early childhood developmental support resulted in normal outcome in most patients. We hypothesize that prompt initiation of early complex rehabilitation in infancy, when neuroplasticity is considered to be the most valuable, in combination with the education and empathetic counselling of the family, might have contributed to the improved long-term outcome. Nevertheless, in patients with intrauterine stroke, the neurodevelopmental outcome was adverse, supporting the idea of the increased propensity of vulnerability of the immature brain. This observation is in comply with the outcome of fetal stroke described in the literature [94]. A better understanding of the risk factors predisposing a fetus to cerebral infarction holds potential for future therapeutic intervention trials.

Based on these initial findings, we designed a population-based study of near-term and term neonates with acute perinatal stroke born over an 11-year period in Central-Hungary. In agreement of the findings in the literature, the overall incidence of acute perinatal stroke was 1 per 1400 live births in our patient population [91, 95]. The incidence of acute *PAIS* was 1 per 3800 live births, the incidence of acute *PHS* was 1 per 5300 live births, while the incidence of *CSVT* was 1 per 11000 live births. The average annual incidence of CHD *and* perinatal stroke in our population was 1 in 11000 live births. Therefore, using the birth prevalence of CHD in Europe [96] approximately one in 90 neonates with CHD is expected to also present with perinatal stroke. This finding underscores the need of awareness for the risk of stroke in infants with CHD.

The finding that 12% of the neonates with perinatal stroke had CHD as well, lends further support to this notion.

One of the strengths of this study was that a high proportion of infants had long-term follow-up up to early school age allowing for detailed documentation of long-term neurodevelopmental outcome in this patient population. Longitudinal examination of neurological outcome data in patients with perinatal brain injury is particularly important because speech and other higher cognitive functions emerge later in childhood [97]. Importantly, in our study, 43% of the patients achieved outcome matching the population norms, underscoring the potential of the developing brain for reorganization.

Nevertheless, in more than half of the patients, the long-term neurodevelopmental outcome was affected. Our data revealed that the interplay of stroke territorial involvement and clinical risk factors influence the long-term outcome of patients with perinatal stroke. In patients with *PAIS*, we have found that main MCA stroke, multiple strokes, the involvement of CST, and infection/inflammation were independent predictors of adverse outcome. Our findings also revealed that developmental disabilities were diagnosed less frequently among neonates with *frontal lobe PHS*. On the other hand, *parietal lobe PHS* increased the risk for CP and cognitive deficit and the involvement of the *thalamus and/or basal ganglia* was also associated with epilepsy. Patients with strokes involving multiple lobes had poor outcome while seizures on admission were associated with the diagnosis of epilepsy beyond the neonatal period. Finally, patients with CHD *and* perinatal stroke were at a significantly higher risk for dying compared to infants with CHD alone. The rate of neurodevelopmental morbidity was higher among neonates with perinatal stroke with or without CHD. This finding suggests that the stroke-associated direct insult to the brain plays the most important role in the development of neurodevelopmental morbidity in these patients.

5.1 Perinatal stroke – from symptoms to follow-up

Our data [78] indicate that in neonates presenting with seizures, following the exclusion of hypoglycemia, electrolyte disturbances, infection, and metabolic diseases explaining

the occurrence of the seizures, we should consider perinatal stroke as the etiology especially if the seizure is of focal onset. The symptoms of perinatal stroke generally present during the first two postnatal days. Nevertheless, perinatal stroke may also present after a normal neonatal period between 4-8 months of age with asymmetric hand preference and/or developmental delay, which is called *presumed perinatal stroke*.

Our study found that a normal cranial ultrasound scan does not exclude the diagnosis of stroke. Indeed, posterior fossa arterial ischemic stroke or stroke in the deep medullary veins are not always detectable on cranial ultrasound. Therefore, the extent of involvement and the vascular territory of PAIS, the presence and location of CSVT and associated infarction and/or hemorrhage can reliable only be confirmed by MRI. The MR techniques most useful in the most appropriate visualization and characterization of the lesion include axial diffusion-weighted imaging (DWI), apparent diffusion coefficient scan (ADCs), susceptibility-weighted imaging, and magnetic resonance angiography (MRA) a list of which may be completed with T2- and T1- weighted sequences [37].

Moreover, the risk factors identified for perinatal stroke themselves may have an impact on the best choice of treatment. For example, if a patient is diagnosed with combined prothrombotic disorder, anticoagulation should be considered especially if an indwelling central venous line is in place, the patient has to undergo complex and or repeated surgical procedures, or the patient suffers from certain comorbidities. Later in life, the use of contraceptive pills and/or smoking is prohibited. In cases with inherited prothrombotic disorders, there are implications for the subsequent pregnancies of the mother and low-molecular weight heparin administration may be carefully considered.

The role of the early, long-term rehabilitation is of high importance. In this case-series, we have described that even in cases of extensive brain damage, normal developmental outcome may be achieved with early childhood developmental support. We have also found that the majority of the patients affected by perinatal stroke did not exhibit long-term neurodevelopmental sequelae. We hypothesize that the more favorable outcome demonstrated in our studies compared to the data in the literature might be, at least in part, due to the utilization of early, active rehabilitation practices. Severely impaired development was observed in the cases of in utero stroke. There are limited data on the

neurodevelopmental prognosis when perinatal stroke is diagnosed antenatally. However, similarly to our findings, the data in the literature indicate that the prognosis is generally poor with high mortality (40%) and long-term morbidity rate [94, 98]. This observation might be explained by the increased vulnerability of the immature brain.

5.2 The role of brain territorial involvement and infection/inflammation in the long- term outcome of neonates with PAIS: a population-based cohort study

In agreement with previous observations [41], we have also found that the volume of territorial involvement is important in predicting adverse outcome [42, 100]. Indeed, large strokes in our study such as involving the main branch MCA and multiple strokes were more uniformly associated with poor outcome. Furthermore, in addition to neurological impairment in most of the affected patients (93%), main branch MCA stroke was also associated with a particularly high rate of CP (87%) compared to patients with other stroke subtypes (8-19%). Nevertheless, these neonates all had a large stroke, with the involvement of the CST, the basal ganglia, the thalamus and the cerebral cortex as well. Therefore, we cannot conclude if the large stroke itself, the involvement of specific anatomic regions, or both has contributed to the development of cerebral palsy/adverse outcome in these patients.

Previous studies have described that the involvement of the CST is associated with poor long- term neurodevelopmental outcome in general and cerebral palsy in particular [36, 40, 100]. Interestingly, and perhaps not quite unexpectedly, other studies have found that concomitant involvement of the cortical and subcortical structures, particularly the PLIC, basal ganglia and cerebral cortex predict hemiplegia.[8] Our study confirms the findings that, after excluding neonates with main branch MCA stroke and controlling for other significant clinical variables, injury of the CST at the level of PLIC with or without the involvement of cerebral peduncles increases the overall risk for adverse outcome and CP/fine motor impairment. Nevertheless, it is of note that 40% of the patients with CST involvement did not have motor deficit in the long- term and 25% of them only had fine motor impairment. Again, these findings likely reflect the immense potential for plastic motor development of the neonatal brain following perinatal brain

injury even when anatomically important parts of the brain are affected.

In agreement with the findings of previous longitudinal studies that found the rate of epilepsy at 10-54% [50, 57, 101, 102], the overall rate of epilepsy was 13% in our study. 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 [102]. 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 [40, 103] and that epilepsy might limit plasticity of the brain during development.[48, 104]

The prevalence of cognitive (17%) and language delay (21%) was also comparable to the findings in the literature (11%-69%) [41, 43] and 10-33% [43], respectively). Our finding that 21% of the patients developed behavioral problems later in life is also in line with reports in the literature (11-59%) [43, 103, 105]. Again, the development of behavioral problems was associated with main branch MCA and multiple strokes. Surprisingly only few studies have investigated the relationship between brain MRI findings and behavioral problems in patients with PAIS [40]. In agreement with the findings of these reports, our observations also suggest that hyperactivity and poor attention are the most common behavioral problems occurring in patients with PAIS [103, 105]. The finding underlines the importance for the need for personalized support and early psychological intervention strategies in PAIS patients with behavioral difficulties.

Although infection has been recognized as a risk factor for neonatal or childhood stroke [106], 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 PAIS. We speculate that the inflammation-associated release of cytokines, chemokines and other local factors might negatively affect brain development in the postnatal period [107]. In addition, infection may also exacerbate prothrombotic tendencies, and the associated endothelial injury along with the release of inflammatory

cytokines could result in decreased thrombomodulin levels and accumulation of tissue factor [14] enhancing the severity of PAIS. Indeed, in newborn dogs, the prothrombotic stress from *Escherichia coli* lipopolysaccharide leads to focal intracranial arteritis selectively in the carotid artery and its branches, and this finding is associated with the development of stroke [108]. In a prospective, international case-control study of childhood stroke, infection also transiently increased the risk of childhood arterial ischemic stroke [106]. There is no clear evidence whether arterial stenosis or vessel irregularity contributes to the development of cerebral infarction [109] or alternative mechanisms such as vasospasm [110] play a role in the development of PAIS 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 [42, 102, 111], 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 [112]. In addition, the ensuing dysregulated release of cytokines, chemokines and local growth factors is contrary to the tightly choreographed and developmentally regulated release of these factors essential for normal brain development. Further prospective studies are needed to delineate the association between PAIS 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 along with a thorough follow-up.

In summary, in line with previous findings [79, 102], we have also found that severe disability and the co-occurrence of impairment in all neurological domains were rare and 43% of the patients had a normal long-term neurodevelopmental outcome. As approximately 30-60% of patients with PAIS develop later CP, current research has primarily focused on improving motor outcomes. Constraint-induced movement therapy and transcranial magnetic stimulation have been shown to improve motor outcome

[113]. Thus, since central nervous system plasticity is likely at maximum in the neonatal period and early infancy [114], prompt initiation of appropriate developmental support, environmental enrichment and parental advocacy are crucial to ameliorate brain injury and to enhance the chances of white matter recovery [115].

5.3 Long-term neurodevelopmental outcome of term neonates with PHS: a population-based study

Recent advances in neuroimaging modalities allow for the prompt diagnosis of PHS and findings on brain MRI may also predict neurodevelopmental outcomes. This is of clinical relevance as early prognostication and parental counseling are of great importance in optimizing supportive care and rehabilitation strategies. In addition, providing cautious reassurance to parents when there is a high likelihood of a good outcome might help in coping with the unfolding events. As a novel finding, none of the patients with *frontal lobe* PHS developed CP, epilepsy, or cognitive impairment during the study period. This finding must be interpreted with caution though, as the status of higher cognitive functions such as emotions, problem solving, impulse control and social interactions regulated by the frontal lobe mostly become evident during school age or beyond. However, in our study, two-thirds of the children with frontal lobe hemorrhage were followed-up until school age. Thus, if the findings on the lack of CP and basic cognitive impairment in children with frontal lobe PHS are confirmed in the future, parents could be reassured about these aspects of the expected neurodevelopmental functions. Of note, two patients with long-term follow-up were lost before the 24-month visit and therefore, they might have escaped the diagnosis of CP.

In most of the patients (60%), neurodevelopmental outcome was significantly affected though. It is important to note that, while long-term neurodevelopmental outcomes were comparable to those of neonates with PAIS [90], the rate of CP was lower (16% compared to 25-50% in PAIS [116]). However, in patients with PHS that developed CP, the condition was more severe as 75% of the infants with CP had tetraparesis. The reason for these findings is unclear. A possible explanation for the increased rate of CP in patients with PAIS might be that, while a large proportion of patients with PAIS had main branch MCA stroke concomitantly involving anatomical structures such as the

basal ganglia, the PLIC, the thalamus, and the cerebral cortex, in PHS such extended involvement was less frequent. Nevertheless, we have found that parietal lobe PHS and PHS affecting multiple lobes were independent predictors for CP. The increased risk for CP in infants with parietal lobe PHS might stem from the fact that nerve fibers in the corticospinal tract partly originate from and/or cross over the parietal lobe. In patients with multiple lobe PHS, the extent of the central nervous system damage likely explains their increased risk for developing CP.

Parietal lobe PHS is not only associated with an increased risk for CP, but children with parietal lobe PHS also had a higher likelihood for cognitive impairment. Again, this finding is similar to those found in PAIS, where the posterior branch MCA stroke and posterior cerebral artery stroke increased the risk for cognitive impairment [40]. The contribution of the parietal lobe to the recollection aspects of episodic memory [117] and language delay [117] may relate to this phenomenon yet exact causative mechanisms need further studies.

Finally, as seen in cases of neonatal central nervous system injury of different etiology such as hypoxic ischemic encephalopathy or PAIS [102], PHS involving the thalamus and/or basal ganglia and multiple lobe PHS increased the risk for epilepsy later in childhood. This is in line with previous findings on patients with ischemic stroke where the risk of developing epilepsy is higher when more substantial damage to the brain occurs [102]. Additionally, we also noted that clinical seizures on admission were associated with epilepsy. In these patients, continuous EEG monitoring and initiation of appropriate early interventions are warranted especially since epilepsy might negatively affect the plasticity of the developing brain [48].

5.4 Mortality and long-term outcome of neonates with congenital heart disease *and* acute perinatal stroke: a population-based case-control study

Very few studies have compared the baseline characteristics and neurodevelopmental outcome of neonates with CHD *and* acute perinatal stroke to those of neonates with CHD without perinatal stroke (Control-1 Group) and infants with perinatal stroke but without CHD (Control-2 Group).

In our population-based cohort study conducted in term and near-term neonates we aimed to assess the impact of the co-occurrence of CHD *and* perinatal stroke on mortality and long-term neurodevelopmental outcome. In line with that data in the literature [4], the average annual incidence of perinatal stroke was 1 in 1400 live births in the present study. The average annual incidence of CHD *and* perinatal stroke in our population was 1 in 11000 live births. Therefore, using the birth prevalence of CHD in Europe [96], approximately one in 90 neonates with CHD is also expected to present with perinatal stroke. This finding underscores the need of awareness for the risk of stroke in infants with CHD. The finding that 12% of the neonates with perinatal stroke had CHD as well, lends further support to this notion. Finally, two-thirds of the patients were diagnosed with cyanotic CHD giving further support for the increased propensity to brain injury seen in children with cyanotic CHD.

This is a unique study as for the first time it compares mortality, clinical characteristics, and neurodevelopmental outcome of neonates with CHD *and* perinatal stroke (Study Group) to neonates with CHD *without* perinatal stroke (Control-1 Group) and patients with perinatal stroke *without* CHD (Control-2 Group). The rate of adverse outcome of neonates with perinatal stroke *and* CHD was similar to that in the study by Cheng et al. with a median follow-up of 15.3 months [56]. However, in addition to investigating the perinatal risk factors in neonates with CHD *and* perinatal stroke, we followed a high proportion of infants for at least 18 months and with a median follow-up age of 61 months. The rate of adverse neurodevelopmental outcome in the Control-1 and Control 2-Groups was also similar to that described in previously published studies [2, 90, 118]. Nevertheless, we have found significant differences in gestational

age at birth, Apgar scores, the number of twin pregnancies, mode of delivery and the frequencies of cardiac interventions between the Study and the Control Group patients. After adjusting for clinically relevant risk factors, mortality remained significantly higher in neonates with CHD *and* perinatal stroke compared to the Control-1 Group patients (CHD only).

The rate of abnormal neurodevelopmental outcome was higher in patients affected by perinatal stroke irrespective of the presence or absence of coexisting CHD: 53% in the Study Group and 52% in the Control-2 Groups compared to 26% in patients with CHD only (Control-1 Group) ($p=0.03$). Thus, normal neurodevelopmental outcome was most prevalent in patients without perinatal stroke. This finding suggests that the stroke-associated direct insult to the brain plays an exceptionally important role in the development of neurodevelopmental morbidity. However, given our finding that a higher proportion of neonates with CHD undergoing surgery develop stroke, the possibility of sequentiality also arises in the context of CHD *and* perinatal stroke. Indeed, studies examining the risk of open-heart surgery or Rashkind septostomy in neonates with CHD found both procedures to be independent risk factors for stroke [119-121]. Our findings that the rate of these procedures was significantly higher in patients of the Study than Control-1 Group and that half of the strokes occurred after surgery, lend further support to this notion in the literature [120, 121].

The rate of C-section was significantly higher in subjects with CHD only compared to neonates with CHD *and* perinatal stroke and with perinatal stroke *without* CHD (89% vs. 39% and 48%, respectively, $p<0.001$). The reason for this finding is unclear, but the high percentage of multiple gestations, the lower gestational age, and the prenatal diagnosis of CHD in most of the patients in the Control-1 Group likely played a role in influencing the obstetric management. Although C-section may offer some protection from the occurrence of neurological deficits [20], it remains unclear whether the higher C-section rate in Control-1 Group patients contributed to the absence of stroke and the higher rate of normal neurodevelopmental outcome in this patient population.

Mortality in infants with perinatal stroke varies between 2% and 25% depending on the pathomechanism of stroke and other clinical risk factors [122]. While mortality in neonates with CHD is around 4% [123], mortality described in the literature for children with both CHD *and* stroke may be as high as 33% [56]. Along these lines, we have also

found that mortality was significantly higher in patients with perinatal stroke *and* CHD (25%) compared to neonates with CHD alone (5%, OR: 6.5 95% CI: 1.1-39.4). Intensive care procedures and especially the use of extracorporeal membrane oxygenation and ventricular assist device are associated with increased mortality in neonates with CHD *and* stroke [54, 56]. Additionally, in previous studies, the use of inotropes or vasopressor-inotropes, the number of cardiac procedures and parenchymal hemorrhage were also associated with mortality in patients with CHD and stroke [56]. The novelty of our study is that after adjusting for neonates with matching CHD or matching strokes only, as well as for the relevant clinical risk factors, mortality was still significantly higher in neonates with CHD *and* stroke compared to patients with CHD only (Control-1). Therefore, although the reasons of the increased mortality of neonates with CHD *and* stroke are incompletely understood, it is tempting to speculate that the coexistence of multiple clinical risk factors synergistically contributes to the increased mortality in this patient population.

In agreement with previously published studies, one-third of the patients with perinatal stroke developed later cerebral palsy in our study [90]. However, our finding that the rate of cerebral palsy was only 16% among neonates with perinatal stroke *and* CHD (Study Group) must be interpreted with caution. Due to the high mortality (25%) in the Study Group, subjects with the worse prognosis including a higher rate of cerebral palsy might have passed away in the neonatal period. On the other hand, we hypothesize that the cumulative effect of delayed microstructural development in combination with multiple ischemic/hemorrhagic events in infants with CHD *and* perinatal stroke, respectively have contributed to the higher rate of epilepsy and cognitive deficit [124]. This finding is in agreement with recent data showing that in children with perioperative neonatal brain injuries, cognitive outcome is worse at school age [125].

5.5 Neonates with CSVT and neonates with fetal stroke

CSVT

Consistent with the available data in the literature, [4] the incidence of CSVT was 1 per 11000 live birth in our investigation. [91] Unlike neonates with PAIS and PHS, patients with CSVT have almost invariably poor outcome. Indeed, 18% of the patients died in the neonatal period, and among the survivors followed for at least 18 months of age, 70% had adverse long-term neurodevelopmental outcome. [91] Three-quarters of the neonates with CSVT had thrombosis in multiple cerebral sinuses. [91] Occlusion of multiple sinuses can cause outflow obstruction and venous congestion, leading to edema, increased intracranial pressure and death [126]. In agreement with the findings of other studies, we found the transverse sinus (82%) to be most commonly affected.[31, 126, 127]

Fetal stroke

Stroke developed in fetal life in 20 neonates (9% of all neonates diagnosed with acute perinatal stroke). Contrary to the findings of Ghi et al [94], none of the patients with intrauterine stroke in our study died in the neonatal period. However, poor long-term neurodevelopmental outcome was demonstrated in 67% of the cases. [91] Three patients with intrauterine stroke were diagnosed prior to delivery on routine ultrasound surveillance, while 17 neonates were diagnosed after delivery as they developed clinical symptoms. In the remaining cases, MR imaging confirmed the antenatal development of the stroke. [91]

Due to the high likelihood of poor short and long-term outcome of patients with antenatal stroke, if the diagnosis is established during fetal life, appropriate parental counseling and guidance are of great importance [94].

The constraints of the framework of the PhD study did not allow for further investigation of the subgroups of patients with CSVT and/or in-utero stroke. However, we plan to conduct further investigations in the near future.

5.6 Strengths and limitations

Our study also has limitations to be considered. First, the analysis utilized, at least in part, retrospectively collected data and was also carried out on data obtained over a relatively long period of time with its inherent disadvantages. Second, due to the uncommon diagnosis of perinatal stroke, 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. Third, only a small number of neonates had prothrombotic test and bleeding diathesis evaluations performed. However, routine testing for the presence of prothrombotic mutations and bleeding diathesis in patients of perinatal stroke have not been suggested [83]. Additionally, due to the constraints of a PhD study, we were unable to investigate further specific subgroups of patients such as patients with CSVT or patients with in-utero stroke. Nevertheless, we intend to continue our investigations on perinatal stroke and hope to characterize these groups of patients in more detail in the near future. Finally, some subgroups with important anatomical lesions were too small to allow for meaningful statistical conclusions to be drawn. As perinatal stroke is a rare condition, thoroughly controlled data collection by regional or national registries utilizing an appropriate oversight structure remains the main avenue for statistically robust data collection, hypothesis generation and testing for treatment efficacy and determining the outcome of neonates with this condition.

Strengths of the study include the large number of neonates enrolled, the confirmation of the diagnosis by MRI in all patients, and the prospective nature of a portion of the follow-up studies. In addition, a large proportion of patients had detailed neurodevelopmental follow-up for at least 18 months of age and many up to early school age. This is particularly important in studies on perinatal brain injury because speech and other higher cognitive functions can only appropriately be assessed later in childhood [97]. Finally, the use of carefully selected control groups in the significantly strengthen the validity of the findings.

6. Conclusions

1. The overall incidence of acute perinatal stroke was 1 per 1400 live births in our patient population. The incidence of acute PAIS was 1 per 3800 live births, while the incidence of acute PHS was 1 per 5300 live births. Both the average annual incidence of CVST and the average annual incidence of CHD *and* perinatal stroke in our population was 1 in 11000 live births. However, despite its rarity in 90 neonates presenting with CHD, one will likely develop perinatal stroke.
2. The interplay of stroke territorial involvement and clinical risk factors influence long- term outcome.
3. Less than half of the patients with perinatal stroke had normal neurodevelopmental outcome.
4. Severely impaired development was observed in surviving patients of in-utero stroke.
5. In cases of PAIS, main MCA stroke, multiple strokes, the involvement of the CST and infection/inflammation were all independent predictors of adverse outcome.
6. Our findings also revealed that developmental disabilities were diagnosed less frequently among neonates with frontal lobe PHS. On the other hand, parietal lobe PHS increased the risk for CP and cognitive deficit and the involvement of the thalamus and/or basal ganglia was also associated with epilepsy. Perhaps not entirely unexpectedly, patients with strokes of any type involving multiple lobes had poorer outcome. In addition, seizures on admission were associated with the diagnosis of epilepsy beyond the neonatal period.
7. Patients with CHD *and* perinatal stroke are at a significantly higher risk for dying compared to infants with CHD only. The rate of neurodevelopmental morbidity was higher among neonates with perinatal stroke with or without CHD. This finding suggests that the stroke-associated direct insult to the brain plays the most important role in the development of neurodevelopmental morbidity in these patients.
8. Neonates with CSVT had an increased mortality and morbidity rate.
9. Finally, we have designed a clinical guideline to aid a more structured approach to the diagnosis and management of neonates with acute perinatal stroke.

7. Summary

Perinatal stroke is an important cause of acquired brain injury in neonates and it constitutes a considerable burden on the affected children, their families and society. Neurological deficits in survivors of perinatal stroke include cognitive impairment, hemiparetic cerebral palsy, language delay, epilepsy, and/or behavioral disorders which often manifest only at school age. Our primary aim was to design a population-based study, involving all patients diagnosed with acute perinatal stroke born over an 11-year period in Central-Hungary and to investigate the long-term neurodevelopmental outcomes of these patients. Our secondary aim was to describe the associations among clinical presentation, clinical risk factors, MRI findings and long-term neurodevelopmental outcome of this condition. The overall incidence of acute perinatal stroke of 1 per 1400 live births in our study is in agreement with the findings in the literature. The incidence of acute PAIS, PHS and CSVT in our study was 1 per 3800, 1 per 5300 and 1 per 11000 live births, respectively. We found that approximately 40% of the infants with acute perinatal stroke had normal neurodevelopmental outcome while the rest of the patients developed chronic neurodevelopmental sequelae. In patients with PAIS, main MCA stroke, multiple strokes, the involvement of the CST, and infection/inflammation were all independent predictors of adverse outcome. Among children with PHS, parietal lobe PHS, the involvement of the thalamus and/or basal ganglia, strokes affecting multiple lobes, and clinical seizures on admission were associated with poor outcome. Patients with CHD *and* perinatal stroke were at higher risk of dying compared to infants with CHD or perinatal stroke *alone*. The rate of neurodevelopmental morbidity was higher in neonates with perinatal stroke with or without CHD. Of note is that patients with CSVT had an increased rate of mortality and severely impaired neurodevelopmental outcome while patients with in utero stroke are at high risk for poor neurodevelopmental outcome. Finally, we designed a guideline to aid the clinician in establishing the diagnosis and managing the care of neonates with perinatal stroke. Since perinatal stroke is a rare condition, rigorously controlled data collection by regional or national registries remains the main avenue for statistically robust data collection, hypothesis generation and testing for treatment efficacy and determining outcome of neonates with this condition.

8. Összefoglalás

A perinatális stroke érsérülés következtében kialakuló szerzett agykárosodás, mely jelentős terhet ró az érintett gyermekekre, családokra és a társadalomra. Az érintett betegek hosszútávú neurológiai károsodásai közé a cerebrális parézis, a kognitív károsodás, az epilepszia és/vagy beszédfejlődési- és magatartás-zavarok tartoznak. Elsődleges célunk volt, hogy populációs-szintű vizsgálattal a Közép-magyarországi régióban felmérjük a perinatális stroke-kal diagnosztizált újszülöttek előfordulási gyakoriságát, klinikai megjelenését és hosszútávú kimenetelét. Másodlagos célunk volt, hogy feltárjuk a lehetséges összefüggéseket a klinikai megjelenés, a rizikó tényezők, az MR képalkotó eredmények és a hosszútávú kimenetel között ezen betegeknél. Az akut perinatális stroke magyarországi incidenciáját a nemzetközi szakirodalom adataival megegyezőnek találtuk (1 az 1400 élveszületésből). Vizsgálataink szerint, az akut perinatális artériás iszkémiás, illetve vérzéses stroke incidenciája 1 a 3800 illetve 1 az 5300 élveszületésből, míg a cerebrális sinus trombózis incidenciája 1 a 11000 élveszületésből. A gyermekek körülbelül 40%-a tipikus fejlődésneurológiai ütemet mutatott, ellenben több mint a betegek felének hosszútávon kóros fejlődésneurológiai kimenetele volt. Perinatális artériás iszkémiás stroke esetén, az artéria cerebri média fő ága területének a stroke-ja, a többszörös stroke, a traktus corticospinalis érintettsége, és a fertőzés/gyulladás fokozta a kóros kimenetel rizikóját. Perinatális vérzéses stroke esetén a parietális lebeny stroke-ja, a talamusz és/vagy bazális ganglionok területének a stroke-ja, a többszörös stroke és az első klinikai felvétel idején megjelenő görcsök álltak összefüggésben a rossz kimenetellel. Azon betegek, akik stroke-kal és veleszületett szívbetegséggel születtek, gyakrabban haltak meg, mint a csak szívbeteg újszülöttek. A hosszútávú morbiditás magasabb volt mind a szívbeteg és stroke-os, mind a csak stroke-os újszülöttekben, a szívbeteg kontrollcsoporthoz képest. A cerebrális sinus trombózisos újszülötteknél és az intrauterin korban kialakult stroke-os újszülötteknél magas arányban találtunk kóros kimenetelt. Készítettünk egy diagnosztikai és kezelési algoritmust mely útmutatóként szolgálhat az akut perinatális stroke-ban szenvedő gyermekek klinikai ellátásához. Mivel a perinatális stroke ritka betegség, regionális vagy országos regiszterek felállítása szükséges a tényeken alapuló adatgyűjtéshez, a hipotézis felállításához, és a betegek kimenetelének előrejelzéséhez.

9. References

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Cerebral sinovenous thrombosis in the neonate. Arch Neurol. 2006 ;63(3):405-9.

10. Candidate's publications

10.1. Publications related to the thesis:

1. **Vojcek E**, Gyarmathy A, Graf R, Laszlo AM, Ablonczy L, Prodan Zs, Seri I: Mortality and long-term outcome of neonates with congenital heart disease and acute perinatal stroke: a population-based case-control study. *Cong Heart Dis*, 17 (4), 447-461. **IF=2.419**
2. **Vojcek E**, Seri I: Perinatalis stroke: Vizsgálati irányelv. *Orvosi Hetilap* 2022, 163 (24), 952-960. **IF=0.707**
3. **Vojcek E**, Graf R, Laszlo MA, Gyebnar Gy, Seri I: Long-term neurodevelopmental outcome of term neonates with perinatal hemorrhagic stroke: a population-based study. *Dev Med Child Neurol* 2022; 64 (8), 971-978. **IF=4.864**
4. **Vojcek E**, Jermendy A, Laszlo AM, Graf R, Rudas G, Berényi M, Seri I: 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. *Early Hum Dev* 2021; 158: 105393. **IF=2.699**
5. **Vojcek E**, Csécsei M, Flach E, Rudas G, Princzkel E: Perinatal stroke - from symptoms to follow-up. [Újszülöttkori stroke - a tünetek megjelenésétől az utánkövetésig.] *Ideggyogy Sz* 2018; 71: 127-136, **IF=0.1**
6. **Vojcek E**, Seri I. A perinatális stroke közép-magyarországi előfordulása populációs szintű vizsgálattal. *Gyermekegyógyászat*. 2022; 73:452-458.

10.2 Publications not related to the thesis:

- 1. Vojcek E, Keszthelyi TM, Jávorszky E, Balogh L, Tory K:** EPG5 c.1007A>G mutation in a sibling pair with rapidly progressing Vici syndrome. *Ann Hum Genet* 2020; 84 (4): 80- 86. **IF: 1,67**
- 2. Vojcek E, Katona A, Nagy D, Rácz K, Túri S, Katona M:** A koronária fisztuláról egy érdekes esetünk kapcsán. *Gyermekgyógyászat* 2012;63 (5) 273-276.o.
- 3. Vojcek E, Baráth Á, Sztriha L, Túri S, Karg E:** Kreatinhiány - a mentális retardáció lehetséges oka. *LAM* 2011;21 (3) 207-211.o.
- 4. Vojcek E, Vojcek L, Kóhalmi L:** A globalizáció egészségügyi vonatkozásai, *Magyar Bioetikai Szemle*, 2008;14 (2) 53-63.o.
- 5. Vojcek E, Kéri S:** Magyarország a határon túl. *Gazdaság és társadalom* 2004; (1) 61-68.o.

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