Universitat de Girona

FINAL DEGREE PROJECT

TCDI vs TCD for stroke risk prevention in children with sickle cell anemia

- A cross-sectional study -

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1. ABSTRACT

Background: Children with sickle cell anemia (SCA) are at increased risk of stroke. Elevated blood-flow velocities in the middle cerebral artery detected by Transcranial Doppler (TCD) are a good predictor of stroke risk in these children. Velocities obtained by TCD are measured by using a specific parameter, the time-averaged mean of the maximum velocity (TAMM). Children with TAMM velocities ≥200 cm/sec are at high risk of stroke, and transfusions as primary prevention might be done.

Transcranial Doppler-imaging (TCDI) is now widely available and it allows the visualization of intracranial vessels.

Few studies have compared the TAMM in TCD and TCDI, and no studies have established a cutoff point for TAMM in TCDI equivalent to the STOP criteria of "normal", "conditional" and "abnormal", which could predict a high risk of stroke in children with SCA.

Objectives: To compare the TAMM velocity obtained by TCDI with the TAMM velocity obtained with TCD in the middle cerebral artery, and to determine a cutoff point for TAMM in TCDI that could predict a high risk of stroke in children with SCA.

Methods: This study is a cross-sectional study of a diagnostic test. 78 children with sickle cell anemia between 2 to 16 years will be evaluated with both TCD and TCDI in order to determinate the TAMM with the two devices. Velocities obtained with both Doppler techniques will be compared using an intraclass correlation coefficient.

Keywords: Sickle cell disease, Sickle cell anemia, Child, Stroke, Transcranial Doppler ultrasonography, Mass screening, Blood flow velocity.

2. ABBREVIATIONS

SCD: Sickle cell disease.

SCA: Sickle cell anemia.

TCD: Transcranial Doppler or non-imaging transcranial Doppler.

TCDI: Transcranial Doppler imaging or duplex.

TAMM: Time-averaged mean of the maximum velocity.

MV: Mean velocity.

MCA: Middle cerebral artery.

dICA: Distal internal carotid artery.

ACA: Anterior cerebral artery.

PCA: Posterior cerebral artery.

STOP: The Stroke Prevention Trial in Sickle Cell Anemia.

CBC: Cell blood count or hemogram.

3. INTRODUCTION

3.1. Background

Sickle cell disease (SCD) includes a group of genetic disorders characterized by the presence of abnormal forms of hemoglobin. This group of disease produces hemolysis and vasooclusive phenomena. The most common and severe of these disorders is sickle cell anemia (SCA) (1)(2).

3.1.1. Sickle cell anemia: overview

Sickle cell anemia (SCA) or drepanocytosis is a type of hemolytic anemia characterized by the presence of an abnormal form of hemoglobin called hemoglobin S (HbS). This mutated form of hemoglobin results from the substitution of a valine for glutamic acid of the beta globin chain. HbS changes red blood cells properties decreasing its solubility and deformability under deoxygenated conditions, resulting into the sickle cell shape. These sickle cells can occlude the micro vascular circulation and cause vasooclusive phenomena and hemolytic anemia (1)(2).

Although individuals affected by abnormal HbS can be both heterozygous and homozygous, SCA only refers to the homozygous form (HbSS), which is the most common and severe form (1), taking a 75% of all SCD (2). The heterozygous form or "sickle cell trait" usually is benign and asymptomatic; in individuals affected by the heterozygous form, abnormal HbS might protect from malaria. That explains the highest prevalence of these disorders in Africa, South or Central America, Caribbean islands, eastern Mediterranean and Middle East populations (3). However, due to population migration, it has been established around the world.

- **Clinical features:**

SCA usually manifests in childhood, and symptoms appear from 6-12 months after birth, once levels of circulating fetal hemoglobin (HbF) had decreased. Vasooclusive phenomena, hemolysis and infections are the main clinical features of SCA (1)(2)(4).

Vasooclusive phenomena

Vasooclusive phenomena result in acute painful episodes that can affect different parts of the body:

- Bone complications:
  - Vasooclusive bone pain, due to multiple ischemic infarcts in the bone trabeculae. Bone pain mainly affects the vertebrae, pelvis and long bones. The most frequent etiology in children is infections.
  - Dactilitys or hand-food syndrome, which is a limited vasooclusive phenomena localized in food and hands. That produces pain and swelling in hands and feet.
  - Avascular bone necrosis.

- Abdominal complications:
  - Crisis of abdominal pain, due to the micro occlusion of abdominal vessels.
  - Hepatobiliary complications, as cholestasis, cholelitiasis, hepatitis or hepatic ischemia.
  - Splenic sequestration, due to a rapid blood sequestration in the spleen that can produce a hipovolemic shock. It can occur in the first weeks of life, and has a sudden onset characterized by abdominal distention, pallor, tachycardia, tachypnea and splenomegaly. It is a life-threatening condition that requires admission to the hospital.

- Acute chest syndrome is defined as the new appearance of an infiltrate with pulmonary symptoms (thoracic pain, hypoxia, respiratory symptoms, and fever).
- Pulmonary manifestations, as hypertension and alteration of the pulmonary function.
- Priapism, due to the micro occlusion of the penile circulation that produces a painful erection.
- Renal manifestations, as hematuria, proteinuria and chronic renal failure.
- Ophthalmic complications, as proliferative retinopathy due to the occlusion of the retinal artery.
- Dermatological complications, due to the micro occlusion of the skin vessels. That produces painful leg ulcers.

- Cardiac complications, as cardiomegaly and ventricular hypertrophy (due to an increased cardiac output secondary to anemia), and myocardial infarction.
- Cerebrovascular complications, as ischemic or hemorrhagic stroke, that will be explained later.

Infections

- Infections, which can cause a sepsis. Infection is a life-threatening condition in children with SCA, and must be suspected in any child with fever >38ºC.

Hemolysis

- Hemolytic chronic anemia, characterized by: splenomegaly, retyculocitosis and increased levels of bilirrubin.
- Acute severe anemia, due to an acute fall in hemoglobin levels. It can be due to splenic sequestration (explained before), or aplasic crisis. An aplasic crisis is a transient detention of eritropoyesis in the bone marrow, which can lead to an acute anemia.

Other manifestations

- Growth and development can be altered in children with SCA. They usually have a delayed growth and sexual maturity, which tends to normalize in adulthood.

❖ **Risk factors to develop a vasooclusive crisis:**

Abnormal HbS has a lower affinity for the oxygen, so the disease can worsen under deoxygenated or stressful conditions, as hypoxemia, dehydratation, infections or fever (1).

❖ **Diagnosis of sickle cell anemia:**

To diagnose a SCA, laboratory tests might be done. The initial blood count must include: hemoglobin S electrophoresis, quantification of HbF and HbA, cell blood count (CBC) and retyculocite determination, coagulation and biochemistry (renal and

hepatobiliar function, ferritin, LDH, haptoglobine and serology of hepatitis B, C, HIV, CMV and parvovirus B19) (2).

Imaging studies may be also useful in children with SCA to identify complications: a chest x-ray in patients with chest paint or respiratory symptoms, a magnetic resonance or nuclear medicine in patients with avascular bone necrosis, an abdominal ultrasound in patients with abdominal pain, or magnetic resonance in patients with neurological symptoms (1)(2).

❖ **Management of sickle cell anemia:**

The goals of the treatment of children with SCA include control and management of complications. Patients affected by SCA should be assessed regularly by a specialized medical team. Education of the disease and genetic counseling is also important.

Base treatment to prevent complications

Primary prevention of complications includes a routine blood test and some medical measures, as:

- Prophylactic penicillin: Should be given to all children with SCA until the age of 5, as primary prevention of infections. Patients with penicillin allergy can receive erythromycin.
- Folic acid
- Vaccines: Children with SCA should receive all the routinely vaccines plus those against Sreptococcus Pneuminiae, Hemophilus Influenzae b, Neisseria Meningitidis C, seasonal influenza and hepatitis A and B.
- It is also important to avoid risk factors of vasooclusive crisis, as hypoxemia, dehydration or infections.

Hydroxyurea

Hydroxyurea is a cytostatic agent used in patients with SCA, so reduces the incidence of acute pain and complications, increasing the survival in these patients. It is indicated en patients with ≥3 admissions for vasooclusive pain per year, or ≥2 admissions for thoracic pain in two years, or 1 episode of acute chest syndrome.

Management of complications

- Infection: Empiric parenteral antibiotics should be started in any child with SCA and fever >38ºC, so an infection is considered an emergency in these children.
- Vasooclusive pain: Should be treated with hydration (oral or intravenous) and analgesia. Blood transfusion may be indicated if the patient is unstable in a very prolonged crisis.
- Splenic sequestration: The goal will be to maintain an euvolemic state by the administration of isotonic solution. If the patient is anemic and hypovolemic, a simple transfusion could be done.
- Stroke: Any child with SCA and stroke should be admitted in the Intensive Care Unit; fluid therapy, oxygen therapy and transfusion should be done, to maintain the hemoglobin S levels of less than 30%. In contrast to standard population, neither thrombolytic nor anticoagulant treatment is indicated.
- Symptomatic anemia and aplasia: Should be treated by hydration (oral or intravenous) and transfusions.
- Acute chest syndrome: Should be treated by hydration, oxygen therapy, empiric antibiotic therapy and transfusion.
- Priapism: Should be treated by hydration and analgesia. If it lasts more than 4 hours, blood aspiration should be done.
- Renal complications: Hydration and IECA are used to treat renal complications.

Curative treatment

The only cure for SCA is the hematopoietic cell transplantation, which can be only realized in children <16years, due the risk of severe complications in older than 16.

❖ **Monitoring children with SCA**

SCA is a chronic disease that may require periodic controls. Control visit are summarized below[1] (2).

[1] See annex 1.

❖ **Mortality and prognosis:**

Individuals affected by SCA survive less than those without SCA. The median age at death in SCA is 42 years for men and 48 for women, although survival may improve with the use of hydroxiurea and the better access to health care.

The mortality in children had decreased after the implementation of prophylactic penicillin and vaccination (5).

3.1.2. Cerebrovascular complications in children with sickle cell anemia and transcranial Doppler screening:

Children with SCA are at increased risk for cerebrovascular complications. The most severe manifestation is stroke, which is defined as "a focal neurologic deficit resulting from cerebrovascular compromise that persists for more than 24 hours, and has neuroimaging evidence of a cerebral infarct corresponding to the focal deficit"(6)(7). Stroke causes substantial morbidity in these children, as residual weakness, spasticity, hemiparesis or neurocognitive deficit (8)(9). It is estimated that 11% of patients with SCA may have a stroke by the age of 20 (10).

The type of stroke changes with age: ischemic stroke is more frequent in children between the ages of 2 to 9, and hemorrhagic stroke occur mostly in adults (10).

In the Cooperative Study of Sickle Cell Disease, the incidence of ischemic stroke per 100 patient years between the ages 2 to 5 years, 6 to 9 years and 10 to 19 years was 0.70, 0.51 and 0.24, respectively. This fact shows the highest frequency of cerebrovascular complications in early childhood (10).

Transcranial Doppler (TCD), an inexpensive and non invasive tool, has become an important technique to identify patients who are at high risk of stroke. The narrowing or occlusion of cerebral vessels can be identified by an increased blood-flow velocity in TCD. Thus, an increased velocity in the cerebral vessels, which is inversely related to arterial diameter, can predict which patients have an increased risk of stroke. The risk

determination in TCD is measured by a parameter called the time-averaged mean of the maximum velocity (TAMM) (8)(11).

Between 1984 and 1994, Adams *et al*. have studied a cohort of children with homozygous SCA who had no previous history of stroke. The objective of this study was to be able to identify children at high risk of stroke by TCD, and choose an optimal cutoff point for blood-flow velocities that may indicate a high risk of stroke (11). A TAMM velocity >200 cm/sec in the middle cerebral artery (MCA) or distal internal carotid artery (dICA) were highly predictive of an increased risk of stroke (40% risk of stroke in three years) (11).

The ability of TCD to identify children at high risk of stroke made possible the development of The Stroke Prevention Trial in Sickle Cell Anemia (STOP), a randomized trial to evaluate chronic transfusions as primary prevention of stroke (9)(12)(13). The STOP protocol used TCD to screen homozygous children with no history of stroke. Based on the TAMM, risk stratification of stroke was interpreted as: “normal”, all mean velocities less than 170 cm/sec; “conditional”, at least 1 mean velocity of 170 to 199 cm/sec with non greater than or equal to 200 cm/sec; “abnormal”, at least 1 mean velocity of 200 cm/sec or higher; or “inadequate” when no readings from the right or left MCA and dICA could be obtained. To enter the study, children had to have two abnormal results on TCD. A total of 130 children were randomly assigned to either receive prophylactic transfusion to maintain HbS to less than 30% of total hemoglobin, or standard treatment. The trial demonstrated a significant difference between the two groups ($p < 0.001$), with a decreased risk of stroke in the transfusion group, that was 93% lower than the standard supportive care group (12).

The STOP study confirmed that TCD could be used as screening tool to identify children at risk of stroke who may benefit from prophylactic transfusion therapy (9)(12)(13). In fact, the incidence of a first stroke had decreased after the implementation of TCD screening, by 0.67 per 100 patient-years in the pre-TCD period, to 0.06 per 100 patient-years in the post-TCD period ($p < 0.001$) (14). TCD has a 86% of sensitivity and a 91% of specificity in the evaluation of stroke risk in children with SCA (15).

Currently, the timing of repeat screening by TCD based on the highest velocity is: for “normal” velocities, screening must be repeat annually; for “conditional” velocities, it must be repeat in three months; and “abnormal” results, it must be repeat in two to four weeks (13). Very low velocities on TCD may be found after an overt stroke (16). (See Table 1).

Result of TCD	Cerebral blood flow velocity measured by TAMM (cm/sec)	Frequency of exam
LOW	70	▪ Repeat after 1 month
NORMAL	<170	▪ Repeat annually
CONDITIONAL	>170 but <200 in the middle cerebral artery and/or distal internal carotid artery. >170 in the posterior cerebral artery or anterior cerebral artery.	
Low conditional	Between 170 and 184	▪ Repeat at three-month intervals. In the case of normal subsequent values, we should adopt the normal conduct for the group.
High conditional	Between 185 and 199	▪ Repeat after 1 month. In cases of two consecutive abnormal results, it is recommended to discuss the risk of stroke and consider a chronic transfusion.
ABNORMAL	Between 200 and 219 in the middle cerebral artery and/or distal internal carotid artery.	▪ Repeat after 1 month. If the value remains ≥200, it is recommended to discuss the risk of stroke and consider chronic transfusion. If the result decreases to 170-199, it is recommended to repeat it in 1 month if high conditional (between 185-199); or 6 months if low conditional (between 170-184). If the result is normalized (<170), it is recommended to repeat annually.
	220 or more in the middle cerebral artery and/or distal internal carotid artery.	▪ Discuss imminent risk of stroke and consider chronic transfusion.
INADEQUATE	When no readings from the middle cerebral artery and distal internal carotid artery could be obtained.	

Table 1. STOP criteria and recommendations for the frequency of TCD according to the result of the examination (13)(27).

However, TCD has some limitations: the machine is not available in most centers, the technique can be hard to learn and inaccurate in poorly trained hands and it is a non-imaging system, which makes difficult the identification of cerebral vessels. Nowadays, modern Transcranial Doppler Imaging or duplex (TCDI) is widely available, and it allows a more accurate identification of vessels (See Table 2) (15)(17)(18)(19)(20). But velocities obtained by TCDI are not validated with those defined in the STOP protocol.

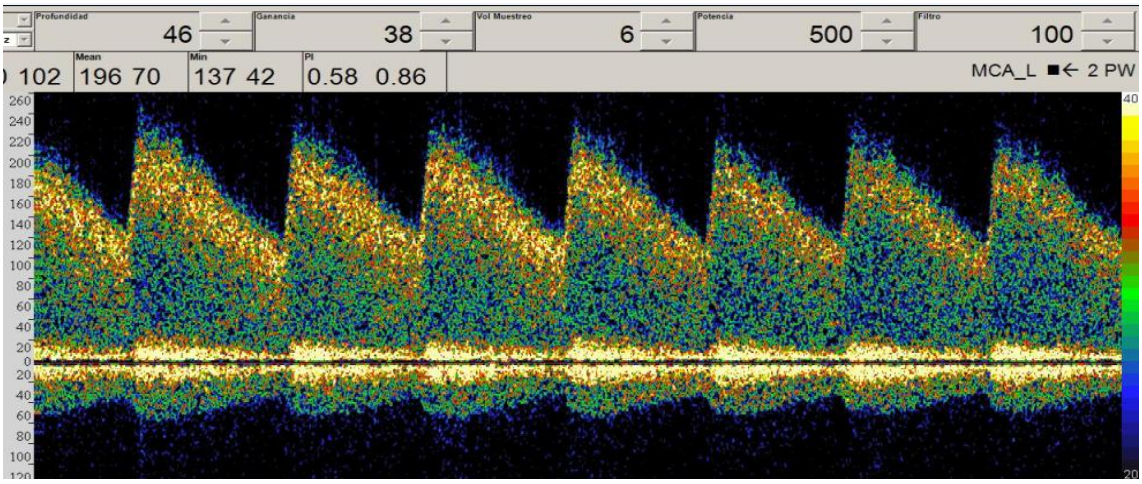

Image 1. Blood-flow velocity of the middle cerebral artery by TCD.

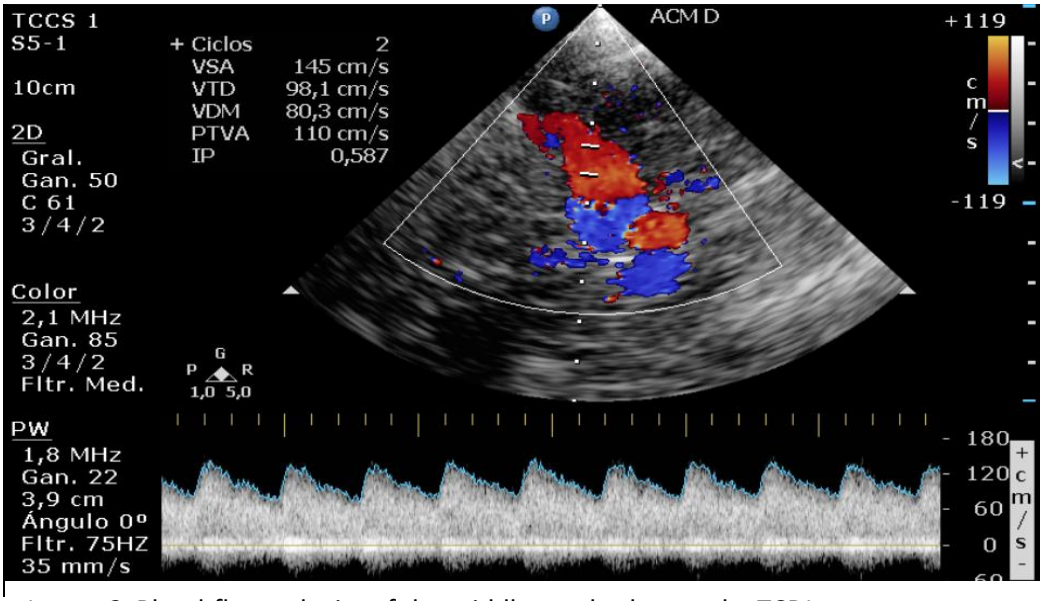

Image 2. Blood-flow velocity of the middle cerebral artery by TCDI.

	TCD	TCDI
Advantages	• It is relatively inexpensive. • It is non-invasive. • It provides a real-time measurement of blood flow characteristics. • The transducer is smallest, and vessel identification may be easy in children with a small temporal window.	• It is relatively inexpensive. • It is non-invasive. • It provides a real-time measurement of blood flow characteristics. • It allows the anatomical identification of the arteries. • It is widely available. • There is a shorter learning curve for the operator. • Other pathology may be identified. • Angle correction is available
Disadvantages	• Arteries are identified "blindly" or without imaging, based on the waveform pattern, depth and flow direction. This requires considerable operator experience. • Inability to visualize intracranial vessels, making arteries identification more difficult or inaccurate. • Limited access to the machine. • There is a long-learning curve for operator. • Angle correction is not available.	• The transducer is heavier, and vessel identification may be difficult in children with a small temporal window.

Table 2. Differences between TCD and TCDI (16)(17)(18)(19)(20)(21)

Considering that the STOP trial is based on TCD, currently it is the gold standard tool and the only recommended for identification of children at high risk that may benefit from primary-stroke prevention by chronic transfusion (8)(13)(21). Nevertheless, TCDI presents some advantages respect TCD that make it interesting to study. Although both TCD and TCDI use the Doppler effect, there are some differences in the devices.
For these reasons, it is important that centers who want to screen children with SCA validate their current equipment with the STOP trial equipment (22). The substitution of TCD by the modern TCDI have to be prudent to avoid variance and an inappropriate selection of children for transfusion therapy (18).

Previous studies have compared both TCD and TCDI to correlate the TAMM velocities with both methods (23)(24)(25)(26), and had demonstrated that velocities obtained with TCDI are from 15% to 10% lower than those obtained by TCD.

However, there are few studies that strongly define a specific cutoff point to predict children at high risk of stroke by TCDI (23)(26), and no studies have established an appropriate cutoff point for TAMM by TCDI to provide comparable velocities to those defined in the STOP protocol.
Moreover, no studies have compared the mean velocity (MV) by TCDI with the TAMM used in TCD.

To further validate these results, we propose to compare velocities obtained with both methods, in order to establish an appropriate cutoff point for TAMM by TCDI to provide comparable velocities to those defined in the STOP protocol that allows the identification of children at high risk of stroke.

3.2. Justification

Cerebrovascular complications are more prevalent in children who have SCA. The most severe manifestation is stroke, affecting 11% of homozygous children by the age of 20 years. Although children usually do not die, stroke has an important morbidity in these children.

TCD is the only tool approved for screening children with SCA to identify those who are at high risk of stroke, which would benefit from transfusion therapy. Some studies have shown that elevated blood-flow velocities of the middle cerebral artery by TCD are predictive of an increased risk of stroke, and for that reason, TCD is now the gold standard test for the evaluation of stroke risk in these children (8)(11)(12)(13).

TCDI is now widely available, and it allows a more accurate identification of the brain vessels in a faster and manageable form. However, the use of different machines may result in different velocity measurements. Few studies have compared the TAMM with the two tools (23)(24)(25)(26), and no studies have established a cutoff point for TAMM in TCDI equivalent to the STOP criteria of “normal”, “conditional” and “abnormal”. Moreover, no studies have compared the mean velocity (MV) by TCDI with the TAMM by TCD.

Because of their familiarity in the ultrasound community, TCDI might be an important alternative to TCD in the evaluation of stroke risk, and may increase the availability of the screening in many centers where TCD is unavailable.

For that reason, we propose to validate the velocities obtained by TCDI with those obtained with TCD using both parameters TAMM and MV, and to determinate a cutoff point for TAMM by TCDI equivalent to the STOP definitions of “normal, ”conditional” and “abnormal”.

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5. HYPOTHESIS

There is a good correlation between the TAMM velocity obtained by TCDI and the TAMM velocity obtained with TCD in the middle cerebral artery, which allows us to use the TAMM by TCDI to predict an increased risk of stroke in children with sickle cell anemia (SCA).

6. OBJECTIVE

6.1. Main objective:

To compare the TAMM velocity obtained by TCDI with the TAMM velocity obtained with TCD in the middle cerebral artery, and to determine a cutoff point for the TAMM by TCDI equivalent to the STOP criteria of “normal”, “conditional” and “abnormal”, which could predict a high risk of stroke in children with SCA.

6.2. Secondary objectives:

- To compare the TAMM and mean velocity (MV) obtained by TCDI with the TAMM obtained by TCD in the middle cerebral artery.
- To assess the MV in the arteries of the Circle of Willis: middle cerebral artery (MCA), distal internal carotid artery (dICA), anterior cerebral artery (ACA) and posterior cerebral artery (PCA).
- To compare the interobserver variability between both TCD and TCDI evaluations.
- To assess the validity (sensitivity and specificity) of TCDI vs TCD.

7. SUBJECTS AND METHODS

7.1. Study design

This protocol is a cross-sectional study of a diagnostic test.

7.2. Study population

The study population will be children with homozygous SCA (HbSS), with the following inclusion and exclusion criteria:

7.2.1. Inclusion criteria

1) Age, from 2 to 16 years.
2) Hemoglobinopathy diagnosis of homozygous SCA (HbSS).
3) A signed informed consent by parents or tutors and child's assent.

7.2.2. Exclusion criteria

1) Other hemoglobinopathies, so are at low risk of stroke.
2) Uncooperative children.
3) Children with a pathological process, as:
   - Fever, defined as axillary temperature above 38ºC.
   - Anemia, defined as hemoglobin levels less than 12g/dl.
   - Any other illness, as vasooclusive crisis, which could increase transcranial blood-flow velocities resulting in an appropriate screening of children. To avoid that, we will perform a basic blood test and constants before Doppler evaluation.
4) Children with an inappropriate transtemporal window.

7.3. Sampling

7.3.1. Sample selection

A non-probabilistic consecutive sampling will be performed with children between ages 2 to 16 years and homozygous SCA. Two centers will be involved in this project: Santa Catherina's Hospital and Josep Trueta's Hospital from Girona. Sampling recruitment will carry out in these two health centers.

Patients will be invited to participate in our study and all of them must sign the informed consent.

7.3.2. Sample size

To calculate the sample size, we will use an adaptation from the library "Sample Size" of the R software (version 3.1.1), created by Marc Saez. Accepting an alpha risk of 5%, a beta risk of less than 20% (statistical power of 80%), the simple size will be **66** patients without dropouts, or **78** patients with dropouts.

Accuracy[1]	**CV 25%**		**CV 50%**		**CV 100%**	
	Without dropouts	**With dropouts**[2]	**Without dropouts**	**With dropouts**[2]	**Without dropouts**	**With dropouts**[2]
1%	49	58	66	78	80	94
2%	20	23	33	38	49	58
3%	10	11	18	21	30	35
4%	6	7	11	13	20	23
5%	4	4	7	8	13	16

Table 3. Simple size
CV 25% small variability of the responses; 50% moderate variability; 100% high variability
[1] Difference (in percentage) that we would like to detect
[2] Up to 15% of dropouts
Finite population: 100 children (annually)

7.4. Variables and data collection

Variables that will be collected are:

7.4.1. Dependent variable:

Main dependent variable:

- The primary dependent variable is the ***time-averaged mean of the maximum (TAMM)*** obtained from de middle cerebral artery (MCA), which is a continuous quantitative variable.

  The TAMM will be measured manually by ***TCDI***, and expressed by cm/sec.

Secondary dependent variables:

- The *mean velocity* (MV) obtained from the arteries of the Circle of Willis (MCA, dICA, ACA and PCA), which is a continuous quantitative variable. It will be measured automatically by *TCDI* and expressed by cm/sec.

7.4.2. Independent variable:

- The independent variable in this study is the ***time-averaged mean of the maximum velocity (TAMM)*** obtained from the middle cerebral, which is a continuous quantitative variable.

  TAMM will be measured manually by ***TCD*** and expressed by cm/sec.

7.4.3. Co variables:

Co variables that will be measured are:

- Sex, which is a nominal qualitative variable. It will be assessed by male / female.
- Age, which is a discrete quantitative variable. It will be assessed by asking the date of birth.
- Hemoglobin concentration, that is a continuous quantitative variable
- Hematocrit, which is a continuous quantitative variable.
- Blood pressure (diastolic and systolic), which is a continuous quantitative variable. It will be assessed by an automatic sphygmomanometer.

To sum up the variables, we have elaborated the table below:

	Variables	Description
Dependent variables	The TAMM of the MCA, obtained by TCDI.	Continuous quantitative variable
	The MV of the arteries of the Circle of Willis, obtained by TCDI.	Continuous quantitative variable
Independent variable	The TAMM of the MCA, obtained by TCD.	Continuous quantitative variable
Co Variables	Sex	Nominal qualitative variable
	Age	Discrete quantitative variable
	Hemoglobin concentration	Continuous quantitative variable
	Hematocrit	Continuous quantitative variable
	Blood pressure (diastolic and systolic)	Continuous quantitative variable

7.5. Measure instruments

To calculate the blood-flow velocities of the brain vessels, we will do a Doppler study with two different devices. We will examine both sides of the head using the transtemporal window to identify the MCA – as the main objective –, and the other arteries of the Circle of Willis (dICA, ACA and PCA) – as secondary objectives –.

Both Doppler examinations will be done at the same day by two different expert neurologists, to avoid interobserver variability. Neurologists won't know the results obtained from each other. Randomization of the two neurologists will be done by the SPSS. Moreover, the Doppler images will be recorded and sent to another center[2] to confirm the results.

The evaluation should be explained to the patient and his parents. The child should be awake and cooperative during the examination, because if the patient becomes sleepy, CO2 level increases and can elevate the velocities, resulting in an inaccurate stratification of stroke risk.

[2] We will send the neurosonographic images to the Department of Neurology of the Medical College of Georgia, the original center from the STOP protocol.

TCD evaluation:

First, we will assess the TAMM velocity with the standard TCD, using the Nicolet TC 2000, Madison, Wis device.

According to the STOP protocol, the highest velocity should be obtained in order to determine the TAMM. The TAMM velocity will be calculate manually, by placing the horizontal cursor so that the area above the line and under the peak of the waveform outline (A) is the same as the area below the line and above the waveform outline (B), and determining it over time[3].

In order to obtain the optimal TAMM, it will be necessary to optimize the Doppler signal in each appropriate depth, which depends on the transtemporal head diameter. According the STOP protocol, transtemporal head diameter is the distance between the posterior aspect of one transtemporal window, to the posterior aspect of the other transtemporal window. Calipers will be used to measure the head diameters. The different depths of insonation depending on the head diameter are specified below[4].

TCDI evaluation:

Then, we will measure the TAMM and the MV with the new TCDI, using a Philips Ultrasound, Bothel, WA 98021 USA device.

The TAMM velocity will be calculated manually, as with TCD.

The MV velocity will be obtained automatically.

7.6. Procedures

Patients involved in our study will be correctly informed about the procedures, and an informed consent must be signed by their parents or tutors before the visit. As explained before, it would be a bi-center project, with the participation of Santa Catherina's Hospital and Josep Trueta's Hospital from Girona. Patients will be cited by phone to come to the visits. The visits will be structured as follows:

- In the first part of the visit, a basic blood test (hematocrit and hemoglobin) and constants (arterial pressure, temperature, cardiac and respiratory frequencies)

[3] See annex 2.
[4] See annex 3.

will be scheduled, to rule out any pathological process that may falsely modify the results of Doppler evaluation.

- In the second part of the visit, the Doppler evaluation will be done. We will assess the blood-flow velocities of the intracranial vessels in children with SCA. We will use both TCD and TCDI devices at the same day. We will determinate the TAMM and the MV velocities, and make a comparison between them to be able to identify an equivalent cutoff point for TAMM in TCDI to those currently used on TCD that could predict which children with SCA are at high risk of stroke.

7.7. Statistical analysis

Sample size calculation is provided in the section 7.3.2 of this protocol.

In the univariate analysis, results will be expressed as mean +/- SD or median (interquartilic range Q3-Q1) for continuous quantitative variables, depending on whether or not they are normally distributed; and as proportions for qualitative variables.

In the bivariate analysis, means will be compared using an intraclass correlation coefficient, overall and stratified by the co variables (continuous variables will be categorized in quartiles).

Finally, we will do the multivariant analysis using a logistic regression, taking as a response the TAMM values obtained by TCDI categorized as below, and as the main independent variable the TAMM values obtained by TCD categorized in the same form as the co variables. TAMM with TCD and by TCDI will be categorized in “normal” and “conditional or abnormal”. In order to assess the validity of TCDI (vs TCD) we will consider TCD as the gold standard. We will estimate validity measures (sensitivity and specificity), and security measures (positive predictive value (PPV), negative predictive value (NPV), positive ratio test (PRT) and negative ratio test (NRT)). Finally, we will estimate the roc curve.

To perform this analysis, we will use the IBM SPSS Statistics 22.0 program. Results will be presented with a confidence interval of 95%.

8. ETHICAL CONSIDERATIONS

This research protocol will be presented to the Comitè Ètic d'Investigació Clínica (CEIC) from Josep Trueta's hospital, and can only be carried out after their approval.

According to the ethical principles from the Declaration of Helsinki and the Law of 15/1999, of 13th December, about "Protection of Personal data", privacy of the patients and data's confidentiality may be protected.

An information document about the study procedures will be given, and an informed consent must be obtained and signed from all the parents or tutors before starting the study[5]. The participant will be also informed.

9. LIMITATIONS

- Doppler evaluation requires previous training, so it is highly operator dependent. This can be a limitation because results may be biased in poorly-trained hands. To avoid this bias, two expert neurologists will carry out the neurosonographic evaluations. Furthermore, Doppler recordings will be send to another center to be reevaluated by another expert.
- Doppler evaluation in children with an inadequate transtemporal window can be difficult and sometimes, the arteries cannot be assessed correctly. This can result in wrong risk stratification.
- SCA is not much prevalent, and that explains why the sample size is small. We will try to solve that by doing a bi-center study, with the participation of Josep Trueta's Hospital and Santa Catherina's Hospital.
- Findings from the secondary objectives will require further study, so this protocol has not been designed to evaluate them.

[5] See annex 4 and 5.

10. WORK PLAN AND CHRONOGRAM

10.1. Staff involved

The staff involved in this project will be composed by:

- Three neurologists (NEUR). Two of them (NEUR1 and NEUR2) will realize the Doppler evaluation in the Josep Trueta's Hospital. The other neurologist (NEUR3) will be from another center and will reevaluate the Doppler recordings sent from the Josep Trueta's Hospital.
- One pediatrician (PED), who will be from the Santa Catherina's Hospital. He will inform the children and his parents about the study. As the way as children will be visit, the pediatrician will inform the parents and the child about the realization of this study, and will give to them the informed consent and the information document to participate.
- One nurse (NSE), who will perform the telephonic citations, the blood test and the assessment of constants.
- One statistic (STY), who will perform the statistical analysis.

10.2. Study stages

The sequence of activities that will be carry out by the research team is detailed below.

1. Coordination stage (4 months)

   -*Activity 1:* At the beginning of the study, a meeting with the research team will be done. We will concrete the objective and define the main variables and the procedures that will be done. The sample sizing will be done according to the inclusion and exclusion criteria.

   -*Activity 2:* Elaboration of the protocol. The protocol will be definitely designed and, before proceed to the second stage, a positive evaluation to the CEIC may be required.

2. Data collection. Exploration of the participants (12 months)

   -*Activity 3: During* this stage, patients and parents will be informed about the procedures by their pediatrician, and an information document may be signed. Progressively, they will be cited by phone and invited to our center.

-*Activity 4:* The visit. During the first part of the visit, a basic blood test will be performed, and constants will be measured. At the second part of the visit, Doppler evaluation will be done with the two devices (TCD and TCDI). It's estimated that the entire visit will last 1 hour.

-*Activity 5:* Data will be collected and included on the database.

3. Data monitoring and analysis (2 months)

   -*Activity 6:* Statistical analysis

4. Publication of results (3 months)

   -*Activity 7:* Final analysis, diffusion of the results and publication of the scientific article.

10.3. Chronogram

To better visualize the work plan, we have created a chronogram [6]

[6] See annex 6

11.FEASABILITY

11.1. Experience of the research team

Doppler evaluation is a diagnostic tool that requires expertise, because is very operator dependent. The experience of our research team with the Doppler technique allows us to perform the examinations faster and with less interobserver variability.

11.2. Availability of resources

In order to develop this study, we have access to the Doppler devices, which are ceded by the hospital; so this saves the purchase of the two Doppler devices.

11.3. Sample feasibility

The sample's recruitment will last about one year, and in order to recruit all the patients that are needed, it will be in conjunction with Josep Trueta and Santa Catherina's Hospital from Girona.

12.BUDGET

The research team (the neurologists and the pediatrician) will perform the tasks related to the extraction of the sample, neurosonographic examinations and data collection. Both devices (TCDI and TCD) will be ceded by the hospital. For that reason, it will not suppose any additional expenses.

Even so, this project cannot be done without:

- Hiring nursing staff, which will realize the tasks of telephonic citation, the blood test and the evaluation of constants. One nurse will be required, and she will cost 20 euros/hous; 39 hours may be required.
- Hiring a statistic, to perform the statistical analysis that will cost approximately 30 euros/ hour, and 72 hours will be needed.
- The laboratory services to analyze the blood samples. Each cell blood count (CBC) will cost 4.44 euros.
- Consumable: A box of 100 CBC extraction tubes will cost 7.09 euros; a box of 100 syringes of 5ml will cost 3.67 euros; a box of 100 extraction needles will cost 1.44 euros; a box of 250 gauzes will cost 15 euros; a bottle of 250ml of alcohol 70º will cost 1.21 euros; and gel for Doppler evaluation will cost 0.42 euros.
- We also need paper to print the information document and the informed consent, and a pen drive to save the images obtained from the Doppler evaluations, in order to be sent to the other center.
- Finally, we have to consider the expenses from attending a congress to present the result (that will cost 500 euros), and the publication costs (that will cost 2,000 euros).

REQUESTED BUDGET	
Expenses	**Euros**
1. <u>Staff expenses</u>	
Neurologists (3)	0 €
Pediatrician (1)	0 €
Nursing staff (1)	780 €
SUBTOTAL	780 €
2. <u>Executive expenses</u>	
Laboratory service (CBC)	346.32 €
TCD device	0 €
TCDI device	0 €
Consumable	29.25 €
Paper	15 €
Statistical analysis	2,160 €
Congress attending	500 €
Publication costs	2,000 €
SUBTOTAL	5,050.27 €
TOTAL	**5,830.57 €**

13.IMPACT OF THE PROJECT

The validation of the TCDI using the TAMM parameter may have an impact in the health system, increasing the number of health centers that will be able to screening children with SCA: because TCDI is more available, the neurologists or radiologists are more familiarized with this technique and it allows a more accurate identification of the brain vessels in a manageable form, a greater number of hospitals will be able to evaluate stroke risk in these children.

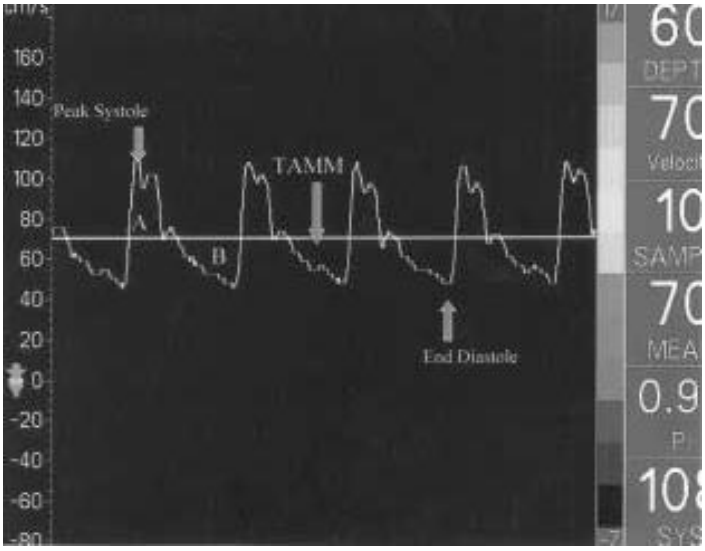
14. ANNEX

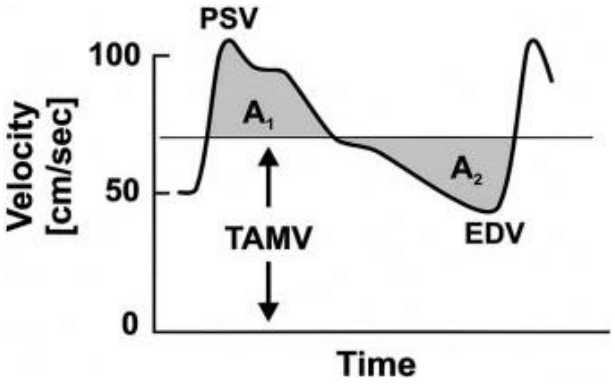
Annex 1: Monitoring children with SCA (2)

	Every 3 months	Every 6 months	Annually	Biannually
Medical evaluation, that includes: • **Anamnesis (pain, jaundice, vaccination…)** • **Physical examination: weight, height and Tanner stage.** • **Blood count** • **If the patient is treated with hydroxyurea: HbF quantification, biochemistry and blood count with reticulocytes.**	In children <1 year.	In children between 1 to 5 years.	In children ≥6 years.	
Psychosocial assessment of school performance			X	
Blood test with retyculocites			X	
Biochemistry. If transaminases or conjugated bilirubin still elevated, a liver biopsy might be done to rule out cirrhosis.				
Ferritin			X	
HbF and HbS quantification			In children younger than 2 years, and a posterior control by the age of 5.	
Simply quantification of urine: in children <6 years.			X	
Macroalbuminemia 24 hours: in children >6 years.			X	
Creatinin clearence			In children older than 12 years.	
Blood pressure and oxygen saturation			X	
Ophthalmologic evaluation: in children older than 8 years.			Annually or biannually	
Transcranial Doppler (TCD)			In children between 2 to 16 years.	

Serology of: HIV, VHB and VHC				Only in children who had received transfusion.
Abdominal ultrasound				In children older than 4 years.
Neuropsychological evaluation				X
Cerebral magnetic resonance (MR)				In children older than 4 years, and if it is normal, repeat between the ages of 8 to 10.
Neurological evaluation				In children with abnormal transcranial Doppler results, lesions in MR or previous stroke.
Audiometry				In children older than 4 years.
Nocturnal pulseoximetriy if there is suspicion of: SAHOS, adenoidal hypertrophy or abnormal TCD.				In children older than 4 years.
Dental evaluation by the deontologist				In children older than 5 years.
Thoracic X-ray				X
Cardiological evaluation				In children older than 8 years.
Spirometry				In children older than 8 years.
Avascular bone necrosis screening: X-ray or Magnetic resonance of the bone				In children with pain or limitation in external rotation from hip and shoulder.
Genetic counseling in adolescents.				

Annex 2: Calculaction of the TAMM velocity (13)(25)





Annex 3: Transtemporal head diameters and expected arterial depths, according to the STOP protocol (13)

Transtemporal head diameters	MCA -1	MCA	dICA	ACA	PCA
12 cm	30-36 mm	30-54 mm	50-54 mm	50-58 mm	40-60 mm
13 cm	30-36 mm	30-58 mm	52-58 mm	52-62 mm	42-66 mm
14 cm	34-40 mm	34-62 mm	56-64 mm	56-68 mm	46-70 mm
15 cm	40-46 mm	40-66 mm	56-66 mm	56-72 mm	50-70 mm

NOTE: MCA -1: distal segment of the MCA; MCA: proximal segment of the MCA.

Annex 4: Informant document to the participants.

FULL D'INFORMACIÓ AL PACIENT

Títol de l'estudi: ***TCDI vs TCD for stroke risk prevention in children with sickle cell anemia***

Ens dirigim a vostè per informar-lo sobre la realització d'un estudi d'investigació en el que se'l convida a participar, de manera completament voluntària. Agrairíem la seva col·laboració en aquest projecte del Servei de Neurologia del Hospital Josep Trueta, ja que la seva participació podria contribuir de manera important a millorar el coneixements actuals sobre el cribatge del risc d'ictus en els afectats de drepanocitosis. Aquest projecte ha estat prèviament aprovat pel Comitè Ètic d'Investigació del Hospital Josep Trueta.

La nostra intenció es que vostè rebi informació de manera correcta i suficient per que pugui decidir si vol participar o no en aquest estudi. Per això,li agrairíem que llegeixi atentament aquest full informatiu i nosaltres li aclarirem els dubtes que puguin sorgir.

També desitgem comunicar-li que aquest treball sorgeix com una iniciativa del Servei de Neurologia del Hospital Josep Trueta, i que es portarà a terme sense rebre compensació econòmica.

A continuació, l'informem sobre la finalitat de l'estudi i els aspectes més importants:

Quina és la seva malaltia?

L'anèmia falciforme o drepanocitosis és una malaltia de la sang en que els glòbuls vermells tenen una forma anormal. Això és degut a la presència d'una hemoglobina anòmala, anomenada hemoglobina S. Una de les complicacions més severes de la seva malaltia és que vostè té més risc de patir episodis d'isquèmia cerebrals, anomenats ictus isquèmics. L'ictus isquèmic es pot prevenir coneixent quins afectats d'anèmia falciforme tenen un risc més elevat, i fent transfusions sanguínies per prevenir-ho quan sigui necessari. Actualment, per tal de poder identificar els pacients d'alt risc d'ictus isquèmics, s'utilitza l'eco-Doppler transcranial, una prova no-invasiva que realitza el radiòleg o neuròleg de forma ambulatòria i periòdica.

Quina és la finalitat d'aquest estudi?

L'objectiu principal d'aquest estudi és comparar dues eines diagnòstiques – l'eco-Doppler transcranial cec, que és la tècnica de referència, i l'eco-Doppler imatge o dúplex, que s'utilitzen en el cribatge del risc d'ictus isquèmic en els nens amb drepanocitosis. Volem determinar quins valors obtinguts amb la nova tècnica equivalen als valors patològics obtinguts amb la tècnica de referència, per poder utilitzar el nou aparell en el cribatge del risc d'ictus isquèmic, donat que és més disponible i permet una exploració més ràpida.

Quines proves es practicaran?

Durant la seva participació a l'estudi, l'informarem dels objectius del projecte i dels dubtes que puguin sorgir.

Les molèsties ocasionades per la seva participació seran mínimes. L'estudi estarà estructurat en una visita:

- Durant la primera part de la visita, li realitzarem una analítica sanguínia que inclourà un hemograma complet. També es realitzarà una presa de constants (temperatura, pressió arterial, freqüència cardíaca i freqüència respiratòria).

-Durant la segona part de la visita, es realitzaran els estudis amb les dues tècniques diagnòstiques. Ambdues tècniques són aparells d'eco- Doppler, probes no invasives i sense efectes adversos pel pacient. Durant l'exploració, vostè haurà d'estar estirat, tranquil i quiet. Es preveu que la durada de la visita sigui d'1 hora aproximadament.

Quins són els beneficis i riscs per participar en l'estudi?

Aquest estudi no comporta cap risc per a vostè, ja que es limita a recollir les dades obtingudes amb els dos aparells d'eco-Doppler per comparar-les posteriorment. Malgrat això, amb la seva participació contribuirà a que, en un futur, pogués ser possible utilitzar el nou aparell –que és més senzill i còmode d'utilitzar - pel cribatge del risc d'ictus en els nens amb anèmia falciforme.

Què passa si decideixo abandonar l'estudi?

Si decideix participar en aquest estudi ha de saber que ho fa voluntàriament, i que podrà abandonar-lo en qualsevol moment sense que això signifiqui cap canvi en la seva

assistència sanitària.

Com s'assegura la confidencialitat i protecció de dades?

Per la realització de l'estudi hem de conèixer algunes dades mèdiques sobre la seva malaltia, així com els resultats de l'analítica i els estudis diagnòstics. Li garantim que les seves dades seran tractades amb absoluta confidencialitat segons la Llei Orgànica 15/1999 de "Protecció de Dades de Caràcter Personal" que regula la confidencialitat de dades informatitzades. Les seves dades seran utilitzades exclusivament en aquesta investigació científica.

Vostè serà informat dels resultats obtinguts en les visites.

Amb qui he de contactar per qualsevol dubte o problema que sorgeixi?

Per contactar amb els responsables de l'estudi, es pot dirigir a:

Dr. .....

Telefòn:.....

Hospital Josep Trueta. Departament de Neurologia

Av/ de França, s/n. 17007 – Girona

Per dur a terme aquest projecte i d'acord amb les disposicions legals vigents, li sol·licitem la seva autorització. En qualsevol moment abans i després de firmar aquest document, del qual vostè es quedarà una còpia, pot preguntar tot el que vostè cregui convenient al personal sanitari responsable.

Declaració del pare/ mare / tutor legal:

Nom del pare / tutor legal: .....

Data: .....

Signatura: .....

Declaració del investigador:

Nom del investigador: .....

Data: .....

Signatura: .....

Annex 5: Informed consentient

FULL DE CONSENTIMENT INFORMAT DEL PACIENT

Títol del estudi: ***TCDI vs TCD for stroke risk prevention in children with sickle cell anemia.***

Declaració :

Jo, ...,pare/mare/tutor legal del pacient..... he sigut informat pel professional de la salut abaix mencionat:

-De les finalitats del present estudi.

-Del procés d'obtenció, emmagatzematge i processament de les dades personals.

-Que la participació és voluntària, que em puc retirar de l'estudi en qualsevol moment i que puc sol·licitar la eliminació de les meves dades personals sense cap repercussió en l'assistència sanitària posterior.

-Que he pogut realitzar les preguntes que he cregut oportunes sobre l'estudi.

-Que he rebut informació suficient per participar en l'estudi.

D'acord amb el que estableix la Llei Orgànica 15/1999, de 13 de Desembre, de "Protecció de dades de caràcter personal", declaro haver estat informat:

-De l'existència d'un arxiu de recollida de dades de caràcter personal.

-De la finalitat de la seva recollida i del responsable de la base de dades.

-De que les dades obtingudes tenen com a únic objectiu la investigació biomèdica.

-De la disponibilitat d'exercir els drets a accés, cancel·lació i oposició a les meves dades en qualsevol moment.

I dono el meu consentiment per participar a l'estudi, consentint l'accés i utilització de les meves dades en les condicions detallades en el full informatiu.

Declaració del pare/ mare / tutor legal:

Nom del pare / tutor legal: .....

Data: .....

Signatura: .....

Declaració del investigador:

Nom del investigador: .....

Data: .....

Signatura: .....

APARTAT PER LA REVOCACIÓ DEL CONSENTIMENT

Jo, ..., revoco el consentiment de la participació del estudi a dalt indicat.

Data: .....

Signatura: .....

Annex 6: Chronogram

	INVESTIGATORS[1]	January 2015	February 2015	March 2015	April 2015	May 2015	June 2015	July 2015	August 2015	September 2015	October 2015	December 2015	January 2016	February 2016	March 2016	April 2016	May 2016	June 2016	July 2016	August 2016	September 2016	October 2016
Coordination stage	ALL																					
Data collection	NSE, PED, NEUR1, NEUR2 and NEUR3																					
Data monitoring and analysis	STY																					
Publication of the results	ALL																					

NEUR: Neurologists; PED: Pediatrician; NSE: nurse; STY: statistical; ALL: all the team.