Non-invasive detection and monitoring of childhood meningitis
(“UNITED Meningitis - Ultrasound Non-Invasive Technology for Early Diagnosis of Meningitis”)

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Method Article

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Abstract

Meningitis can be a life-threatening disease if not promptly diagnosed and treated. Among infants and neonates with a permeable fontanelle, clinical presentation is usually unspecific, justifying the need for an invasive procedure such as lumbar puncture (LP) in order to rule in or out meningitis. This investigation aims to validate a non-invasive, ultrasound-based transfontanelllar device to screen for meningitis by counting WBC beneath the anterior fontanelle. A customized ultrasonic probe working at 20mHz will be used to detect white blood cells, and deep-learning models will be trained for classification. The proof-of-concept study will include 16 participants, and sensitivity and specificity of the device will be calculated. The study will be performed in three Spanish Hospitals, and will include children under 12 months old, a permeable fontanelle, suspected meningitis and a LP performed within 24h before recruitment. The expected time to reach the pre-defined sample size is 18 months.

Introduction

A.1 General points

A.1.1 Introduction

The CIP contains all the items listed in accordance with Appendix A of UNE EN ISO 14155:2021 or refers to the documentation where the required information can be found. For example the Investigator’s Manual.

A.1.2 Identification of the clinical investigation plan

a) name of the clinical investigation;

Non-invasive detection and monitoring of childhood meningitis

b) reference number that identifies the specific clinical investigation, if available;

There is an internal Clinical Investigation code: UNITED Meningitis

c) CIP version or date; Rev 03 Jan/2022

d) summary of revisions in the case of amendments;

A “Change control” box has been included on page 1 of this document to list the record of amendments. Furthermore, in order to identify the sections where changes have been made, a sidebar on the right of the document has been included (as in this section).

All revisions to the protocol have been approved by the CEIC (Clinical Research Ethics Committee) and the AEMPS (Spanish Agency of Medicines and Medical Products) through amendments.
e) version/edition number and reference number, if available, with page number and total number of pages in each page of the CIP

Rev 03, total number of pages = 38

A.1.3 Developer

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A.1.4 Principal investigator, coordinating investigator and research centre(s)

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b) Centres where the clinical research will take place and participating researchers

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A.1.5 Comprehensive synopsis of the clinical investigation

Synopsis:
Each day, 450 children under the age of one die of meningitis and 30% of survivors have lifelong neurological after effects. Meningitis an infection that may present with only fever but can cause death within hours. For this reason, early detection is essential. As a current diagnostic technique, an invasive and potentially dangerous lumbar puncture (LP) is performed to extract a cerebrospinal fluid (CSF) sample and is the only method available to test for the disease. An increase in the white blood cell (WBC) count in the fluid should prompt immediate treatment. However, in industrialised countries, 95% of lumbar punctures in infants with suspected meningitis are negative, resulting in unnecessary hospital costs of 1.5 billion euros in the EU alone. While in developing countries with a lack of laboratories, LPs are rarely performed, with 50% of newborns with meningitis dying from the disease. There is a global need for non-invasive detection of infant meningitis.

Objective:
Our objective is to test the performance of a new non-invasive system (Neosonics) based on high-resolution ultrasound (US) to detect infant meningitis. An ultrasound produces an image of the white blood cells in the CSF through the fontanelle and counts the cells using an algorithm. This detects meningitis and will hopefully reduce the number of LPs, as well as hospital costs.

In addition, it is also proposed to use the device to monitor the patient’s response to treatment through a non-invasive measurement of the white blood cell count, which decrease with a positive response to treatment.

Methodology:
The methodology of the study has been designed in 4 phases, as set out below.

Objective:

Subjects

Sample size

Device

Participating centre

Phase 0

Exploratory
To test the variability in the thickness of the fontanelle

Newborns and infants with an open fontanelle

40

Toshiba Aplio 400/500

HULP and HUQM

Phase 0-NS Exploratory with NS

To detect anatomical structures, improve segmentation of images to be able to apply the cell counting algorithm and facilitate the handling of the equipment

Newborns and infants with an open fontanelle.

40

Standard ultrasound scanner + Neosonics

HULP and HUQM

Phase 0-NS- HSJD

20

Standard ultrasound scanner + Neosonics

HJSD

Phase 1

Concept test

Preliminary assessment of the level of diagnostic agreement between methods

Newborns and infants <1 year or with the fontanelle open and require a LP.

16 (6 with meningitis)

Ultrasound scanner + Neosonics and

LP

HULP, HUQM and HSJD
86 (37 with meningitis)
Neosonics + LP
CISM and HCM
(Mozambique, Phase 1-MOZ)

Phase 2

Sensitivity and specificity

Level of diagnostic agreement between methods

Newborns and infants <1 year or with the fontanelle open and require a LP.

170 (77 with meningitis)
Standard ultrasound scanner + Neosonics and
LP
HULP, HUQM, HSJD and HER.

Treatment monitoring performance

Standard ultrasound scanner + Neosonics

Duration

Phase 0 was completed in Q1 of 2020 (see “Phase 0 Results”). Subsequently, with the appearance of Covid-19, patient recruitment to the trial was delayed due to hospital overload.

Eventually, after the inclusion of HSJD in the study (Rev 02 Dec/2020) the recruitment for Phase 0-NS was completed in June 2021.

Below is a diagram that summarises the length of the study phases and the dates they occurred (approximately)

2020
2021
2022
2023
Dec
A.2 Identification and description of the investigation product

a) summary description of the investigation product and its intended purpose;

This study has been designed in four phases, in which two different devices are used: a standard ultrasound machine (Toshiba Apio 400/500, GE Logiq S8 or another with similar features) which is used to obtain transfontanellar images (Phase 0); although it will also be used as a support device during the rest of the acquisitions to provide greater safety for the users in the positioning of the probe (the entire study), and the Neosonics device; a device in development and the reason for the clinical trial, and used in Phases 0-NS, 1 and 2.

The standard machine used for transfontanellar ultrasound is an ultrasound system that is intended to be used for the visualisation of any kind of structures, features and dynamic processes of the body to provide images that can assist with clinical diagnosis. These devices are certified with EC marking and in fact have already been introduced in the hospital and are routinely used to obtain transfontanellar images. Technically, different transducers can be used in both devices, but in this particular case the transducer to
be used is the one that allows a maximum acoustic intensity of 14 MHz. Structures of up to 300 µm can be seen with this signal, but are not sufficient to view white blood cells.

The prototype (Neosonics) is based on a device marketed by Cortex Technologies ApS (Denmark) and is CE/FDA marked for use in clinical dermatology. Neosonics has similar but improved technology to achieve greater sensitivity. In particular, the maximum safe sound intensity levels for use in dermatology are (16 mW/cm²) and for Neosonics they will be less than 80 mW/cm²; in both cases lower than those of neonatal ultrasound.

The system consists of a base and a probe. The base includes the transformer, the power supply unit, the electronic signal, the motor control system, the motor signal filter and data transmission via USB.

The probe allows the performance of the measurement and includes the sensor, the mechanical system that scans the transducer and the electronics to activate or stop the mechanical system as well as to transmit and receive the sensor’s electrical excitation and reception signals, respectively. The sensor’s working frequency is 20 Mhz, the focal distance is 16–20 mm and the skin penetration is between 4 and 6 mm. The scanning length and speed are 12.1 mm and 1–5 frames/second, respectively. The sensor’s axial travel is 7 mm. The transducer inside the probe does not come into contact with the skin, however, to ensure signal propagation with as little attenuation as possible; unlike other ultrasound scanners, the transducer is in contact with a hydrogel (consumable). The consumable is disposable and is replaced after each use, minimising the risk of contamination between patients and sensor deterioration. The consumable is attached to the probe with a tip that is part of the kit (inventory). The use of this hydrogel not only allows signal attenuation, but also avoids the need to use ultrasound gels with the equipment, facilitating the attachment of the device to the eyelid.

In Phase 0-NS and in Phase I, the probe must be connected to a laptop computer on which the visualisation and storage software must be installed. The visualisation on the laptop allows the user to detect the fontanelle structures and to position the measurement centre in the cephalic fluid space. This procedure gives the user trust in the device when the measurement is performed automatically. It is expected to be able to integrate the automation of the measurement in Phase I (final) and in Phase 2, where the processing on the laptop will be integrated into the base, thus eliminating the need to display and interpret an image.

The counting method first detects the echoes and inserts them into the image. For this, spatial filtering is performed to reduce acoustic and electronic noise using algorithms widely used in medical image analysis. Finally, a count of the detected echoes and a depiction of the range, width and frequencies for their classification is performed, if possible. For this, the images obtained with the investigation equipment are anonymised at the time of acquisition and transferred to NBS using an encrypted hard drive or uploaded to a password-protected server. The NBS data analyst runs the algorithms and provides the researcher with a result of the cell count present in the cerebrospinal fluid. In Phase 2 (efficacy), the algorithm is run on the device immediately.

b) information on the manufacturer of the investigation product;
The manufacturer of Neosonics is New Born Solutions (NBS). NBS is a spin-off company that originated from Madrid-MIT, a community of Madrid initiative in partnership with the Massachusetts Institute of Technology to leverage and boost the industry and biomedical research in the Madrid region. The M + Visión researchers are selected to form multinational and multidisciplinary teams to generate ideas, solutions and international collaborations that lay the project foundations. A result of this initiative was NBS’s creation of an office that has worked on the design and development of this technology, with the objective of launching on the market in 2020, to improve the provision of a new tool for non-invasive detection of meningitis.

NBS is implementing a Quality Management System (QMS) based on ISO 13485:2016 and is working with ISO 13485:2016-certified contractors or suppliers of electronic/mechanical components certified with EC marking.

The NBS office is located at: C/Duque de Medinaceli 2, 1a 28014 Madrid, Spain

The NBS operating unit is located at:

Parc Científic de Barcelona
C/Baldiri Reixac 4-12
Cluster II Planta 2, C082 08028, Barcelona, Spain

c) model/type name or number, including software version and accessories, if available, for full identification;

The name of the device is Neosonics.

The current device requires a computer to take images of the fontanelle and perform cell measurement.

It also consists of consumables that encapsulate the water in contact with the transducer and prevent it from leaking out of the device during use. This consists of a PVA membrane that is attached to the top of the probe with a ring.

In Phase 2 (efficacy), the study will be carried out with Neosonics-3, which already has integrated counting algorithms and does not require a laptop, so the user does not need to view the fontanelle or the measurement site. The equipment automatically performs the image segmentation procedure and informs the user if they need to move the probe or not, and the equipment automatically places the focus on the area of interest (cerebrospinal fluid).

d) description of how traceability is achieved during and after the clinical investigation, for example, by assigning batch numbers, or serial numbers;

Traceability is confirmed by the assignment of serial numbers (s/n) to each of the devices that is manufactured. Each s/n provides the manufacturing date and the component traceability, and the device
validation via a production control record. This record forms part of the NBS QMS.

Regarding the consumables, traceability is achieved by using batch numbers, which identify the components, person responsible for the manufacturing and validation.

Neither the device nor the consumables need to be sterilised.

e) intended purpose of the investigation product in the proposed clinical investigation;

To test the performance of the device (Neosonics) to detect infant meningitis non-invasively by counting white blood cells in the CSF through the fontanelle. For this, the results will be compared with the patient’s diagnosis from their clinic, biochemical, viral and bacteriological analyses, in addition to the CSF white blood cell count obtained by the current method (lumbar puncture).

In addition, it is also proposed to use the device to monitor the patient’s response to treatment through a non-invasive measurement of the white blood cell count, which decrease with a positive response to treatment.

f) The populations and indications for which the investigation product is intended; There are two types of population depending on the study Phase:

- Phase 0 and 0-NS: Premature newborns, term newborns and infants less than 12 months or older who still have an open fontanelle and who may or may not have signs of meningitis.

- Phases 1 and 2: Newborns and infants less than 12 months or older with an open fontanelle and who require a lumbar puncture.

g) investigation product description including any material that will be in contact with tissues or body fluids. (This should include information on any medicinal product, human or animal tissues or their derivatives, or other biologically active substances);

As described in the Investigator Manual, the intended use of the Neosonics device entails the only active point of contact of the device with the patient being the tip of the probe. The rest of the probe will be operated by the user to ensure the correct placement of the tip on the patient’s skin.

The other parts of Neosonics (base and computer, the latter in Phase 0-NS and 1), are connected to the probe by a wire, located at a particular distance, so it does not come into contact with the patient.

Accordingly, the consumable material located at the tip of the probe has been chosen to avoid any potential biological risk to the patient. Therefore, the materials have been selected taking into account UNE EN ISO 14971:2020 (Risk management) and UNE EN ISO 10993-1:2010 (Biocompatibility).

In accordance with the applicable regulations, the features of Neosonics must be considered:

- This is a non-invasive device
- It only comes into contact with the skin of the patient located in the infant or child's fontanelle

- Transient patient use, intended for continuous use of <60 mins.

h) summary of training and experience necessary to use the investigation product; Emergency department doctors, paediatricians and neonatologists, as well as nurses who deal with newborns and infants. In phases 1 and 2, specialists should have experience in recognising signs of meningitis and in performing LPs.

i) description of specific medical or surgical procedures used with the investigation product.

The procedure performed with the device is different between the exploratory phase (0) and phases: 0-NS, 1 and 2.

- Phase 0: Transfontanellar ultrasonography is performed using the device certified and marketed by Toshiba; the Aplio 400/500. The high-frequency ultrasound equipment selected for the study and the images are transferred to a laptop computer. The examination preparation does not take more than 10 minutes; the scanning time will also not exceed 10 minutes. For this, the probe is located on the skin in the fontanelle area in those patients with a fontanelle that is still open and a visualisation of the transfontanellar space is performed. These images are used to improve the algorithm that Neosonics uses in Phases 1 and 2 for the measurement of white blood cells. In particular, the different layers that compose the fontanelle (skin, dura mater, arachnoid, CSF, pia mater) are classified by an imaging expert. The images are used to take manual measurements of the layers using visualisation software (for example, ImageJ). The thickness of the different layers at the tissue interfaces is defined by an orthogonal digital line. This information is used to calculate the tissue attenuation.

- Phase 0-NS: The management of the patient is the same in this phase as in Phase 0, however, in addition to performing the transfontanellar ultrasound with the standard equipment available (Toshiba Aplio 400/500, GE Logiq S8 or other), when completed, an acquisition will also be carried out with the investigation equipment (Neosonics). Neither does the examination preparation take more than 10 minutes; nor the investigation time with both devices exceed 30 minutes. For this, in both cases, the probe is located on the skin in the fontanelle area in those patients with a fontanelle that is still open and a visualisation of the transfontanellar space is performed first with the standard ultrasound machine and then with the investigation equipment. The established guide should be followed for the positioning of the probe. The aim of these images is to improve image segmentation and the transfontanellar space structure recognition algorithm to be used with Neosonics in Phases 1 and 2 to measure white blood cells, as well as to facilitate the equipment handling by the researchers.

- Phases 1 and 2: The patient management in these two phases is the same as that outlined in the hospital for lactating newborns needing a LP. In this case, the lumbar puncture procedure follows the current hospital protocol. The CSF analysis will be carried out in the Emergency Laboratory (Central Services), in accordance with the hospital's standard practice. The CSF cell count procedure is performed by optical
microscopy using the Fuchs-Rosenthal chamber. The analytical objective of the laboratory is a variable interobserver of 15% and 5%, (<30 cells/μL) for low cell count and (>30 cells/μL) for high, respectively.

However, in parallel to the puncture, an examination of the CSF using a standard ultrasound machine and with Neosonics is performed blinded to the results of the LP, to obtain an image of the white blood cells and to be able to measure the number and check the specificity and sensitivity of the probe according to the LP using the algorithms.

If LP-confirmed meningitis cases are recruited, the Neosonics acquisition, which will be blinded to the LP results, should be carried out as soon as possible once the LP results are available, preferably within 24 hrs.

To examine the CSF using Neosonics the same procedure as with a standard ultrasound is followed. The examination preparation does not take more than 10 minutes; neither will the scanning time exceed 20 minutes. For this, the probe is located on the skin in the fontanelle area in those patients with a fontanelle that is still open and a visualisation of the transfontanellar space is performed. In Phase 1, with the assistance of the computer and special software, the user can view the image obtained and is guided where to position the probe on the measurement area. Image acquisition at this stage is done in the research phase and once the strobe has been placed on the measurement area. The images will be stored in the equipment and later transferred for analysis.

In Phase 2 (efficacy), the equipment automatically places the strobe on the area of interest and will execute the algorithm in situ, providing the user with a cellularity result (Neosonics-2).

In addition, in those patients being tested for meningitis who are receiving specific treatment, investigations are performed every 24 hours during the first three days and every 48 hours until the end of treatment or the device does not detect cellularity, to see if it allows the observation of the reduction in the number of white blood cells. In accordance with the standard practice, no follow-up LP is performed on these patients, except on discharge, unless specified by the clinic. Therefore, not all the data is comparable with the results obtained from the LP, but they are of important clinical value for the follow-up of this type of patient.

A.3 Rationale for the clinical investigation design

The rationale for the clinical investigation design, should be based on the clinical investigation results, as specified in section 5.3, and should comprise:

a) an assessment of the results of relevant preclinical trials/studies performed to justify the investigation use of the product in human subjects, and

Bacterial meningitis is an invasive infection of the central nervous system that causes significant morbidity and mortality in newborns and young infants in both developed and developing countries [1].
Its occurrence in developed countries is 0.22/1000 live newborns, with a mortality rate of 10% and the prevalence of permanent neurological sequelae is up to 50% in survivors [1]. The actual occurrence in developing countries may be underestimated, mainly in areas with poor resources, due to the difficulty in diagnosing meningitis and insufficient access to health resources. The mortality rate is much higher in these countries, being approximately 40-58% [2].

The clinical presentation of meningitis at this age is non-specific, with signs and symptoms often being subtle. To confirm the diagnosis, a lumbar puncture is required to obtain cerebrospinal fluid (CSF) for culture and the quantification of cellularity, as well as certain relevant biochemical parameters such as protein or glucose concentration, among others [3]. This is an invasive procedure which is often traumatic at this age (estimated incidence of traumatic lumbar puncture 30-46%), leading to sample contamination with blood, which makes it unreliable. The clinical situation of the patients, which is often unstable or with signs of coagulopathy, makes the procedure impracticable or clearly contraindicated [4]. Furthermore, lumbar puncture may be the cause of a meningeal syndrome (headaches and meningismus) that masks the development of the clinical picture. In addition, CSF analysis is often problematic in developing countries due to the lack of suitable laboratories, so the diagnosis relies solely on clinical suspicion.

Clinical protocols for the diagnosis of meningitis are based on a high index of suspicion, which leads to a high percentage of lumbar punctures in children for whom the final diagnosis is not meningitis. Thus, it has been reported that in up to 95% of lumbar punctures performed due to the suspicion of meningitis the final diagnosis was not meningitis. This signifies a very low output for the procedure, since lumbar puncture does not provide any patient care benefit in a large percentage of the target population. This would justify exploring other diagnostic methods for suspected meningitis that are easy to use in any geographic area and whose accuracy is comparable to traditional analytical methods. We consider safety to be essential in the alternative, given the low prevalence of the disease and therefore, the high rate of unconfirmed suspicions.

Previous studies have used transfontanellar ultrasonography for the non-invasive assessment of CSF cellularity using a 5-7 Mhz probe [5]. This is a feasible method that, however, showed low sensitivity when the CSF cell concentration was less than 500 cells/μL. More recent studies [6,7] showed that the use of ultrasound at frequencies at or above 20 Mhz or higher allows the detection of cells in fluid suspension individually in vitro, even at very low concentrations (10 cells/μL). However, there are no more pre-clinical or clinical studies as this is a novel technique.

Our group has replicated the previous study [6,7] using animal skin to more faithfully replicate the clinical scenario, which entails the attenuation introduced by the biological tissues covering the fontanelle. The study [8] used a high-frequency ultrasound (HFUS) device to detect white blood cells in the CSF in vitro, at concentrations relevant for meningitis diagnosis and with more detailed accuracy than standard manual counting using the Fuchs-Rosenthal chamber. The white blood cell concentrations in a simulated CSF model, in the range of 0-50 leukocytes/ml, were tested and compared with the standard method. Within this range, excellent consistency was noted (Cohen's kappa [k]=0.78-90) between HFUS and manual counting. In fact, with HFUS, concentrations of up to 2 cells/μl were detected.
The promising results obtained by using HFUS and the lack of previous studies reported of a similar sort have prompted the need to perform clinical trials in order to verify the efficacy of the device in a real clinic and thorough the fontanelle.

- Despite the innovation in technology, the safety of the device is indirectly demonstrated by its equivalent device manufactured by Cortex and used in dermatology (Dermalab), as well as by other similar devices such as Toshiba’s Aplio 500. Neosonics, like a standard ultrasound scanner, is a Class IIa device that comes into contact with the patient’s skin for a short period of time and does not test physiological vital parameters. In addition, in patients with suspected meningitis, the patient management and diagnosis are governed by the results of the LP (currently established hospital protocol). With Neosonics, an additional measurement is simply performed to compare methods, so the study as such cannot be considered interventional at clinical management level.

- However, to fine tune the device, a research project is proposed, which consists of 4 phases:

  o **Phase 0**: Preliminary research study to obtain images of the fontanelle with the Toshiba Aplio 500 device in 40 patients. These images will allow the improvement of the measuring device algorithm, as well as the read-out area.

  o **Phase 0-NS**: Research study with the investigation equipment to detect the structures seen in Phase 0, but at high resolution and to facilitate the handling of the investigation equipment. The study is planned for 40 patients recruited at the HULP and the HUQM and on 20 patients at the HSJD.

  o **Phase 1**: To assess clinical safety, a Phase 1 proof of concept study will be carried out in 16 (6 with a LP-confirmed diagnosis of meningitis) patients. After improving the sensitivity of the equipment and the algorithm, Phase 2 will be performed.

  o **Phase 2**: In this phase the sensitivity and specificity will be tested, as well as the consistency of the cellular result of the equipment versus the manual count in 170 patients.

In both phases (1 and 2), the patient will undergo a lumbar puncture (according to the clinical criteria) so the use of the device does not alter the procedure.

The appearance of any Serious Adverse Effects (SAE) will be assessed in all phases by an independent committee; although no potential risks are expected from the use of the device.

b) an assessment of the clinical data that are relevant to the proposed clinical investigation. There are no previous publications demonstrating the use of Neosonics in clinical practice, which is the reason why authorisation is requested for this clinical trial.

A study has been published (previously mentioned) on the current sensitivity of the probe, with in vitro results, and there are publications on infant meningitis and its diagnosis by Lumbar Puncture. Some of these publications have been referenced in the previous section (A3).
A.4 Risks and benefits of the investigation product and clinical investigation

a) Expected clinical benefits;

No direct benefits are expected for the patient during the development of the study. However, an improvement in patient care is expected when the device is introduced into clinical practice compared to the current method and management.

There are no direct expected benefits from this study, as patient management will depend on the results of the LP and the results obtained with Neosonics will be compared with those from the LP, but will not be diagnostic.

However, we believe that the introduction of non-invasive tools in the diagnosis of newborns and infants is essential, given the enormous vulnerability of this target population. Patient participation in the study may help advance knowledge about the capabilities and role of high-frequency transfontanellar sonography in the diagnosis of cerebral pathologies such as meningitis. The current alternative to the method we are proposing for the diagnosis of changes in the cellular composition of CSF is an invasive test, lumbar puncture, which is not always reliable or tolerable by the patient, and can cause them direct or indirect complications, which is why use of the device in the future could result in:

- A 95% reduction in lumbar punctures for patients who are meningitis-free

- A reduction >90% of time spent on lumbar punctures (decision, parent information, preparation and performance: 60 minutes).

- Early detection of the presence or absence of meningitis in patients where lumbar puncture is contraindicated.

- Follow-up of treatment with the device and always depending on the clinical condition can reduce medication and hospitalisation of the patient.

- Early referral of non-critical patients to the ward, always depending on the clinical condition

- Earlier assessment of the response to treatment with antibiotics

- Savings in hospital costs estimated at 5-7% of the costs in Neonatology/Paediatrics.

b) expected adverse effects of the product;

No different adverse effects from those that can be seen in the conventional diagnosis and treatment of meningitis are expected.

Ultrasounds have been used safely in clinics for over 20 years. It is a technology that uses non-ionising radiation, so it does not have the same risks associated with X-rays or other types of ionising radiation. As
long as it does not exceed the safety limits established by the regulatory agencies (EC and FDA) ultrasound does not cause any adverse biological effects in foetuses, newborns or adults [9]. This safety limit is set at a delivered intensity (ISPTA,3) of 94 mW/cm² for transfontanellar ultrasonography. The ultrasound system for the study is certified and used in the dermatological clinic. Therefore, the system operates at 20 MHz and is limited to an output intensity below the set maximum.

The discomfort due to the ultrasound scanner is minimal and may be due to the contact of the transducer and gel with the skin, as can happen with an ultrasound scan. However, the acquisition procedure will be performed by experienced health care professionals who take transfontanellar images routinely in clinical practice.

c) risks associated with participation in the clinical investigation;

No different risk from the conventional management, as the diagnosis and management of the patient is dependent on the results obtained with LP; a technique that is now established in routine clinical practice.

d) possible interactions with concomitant medical treatments; There are no interactions.

A.5 Clinical investigation objectives and hypothesis

a) primary and secondary objectives and hypothesis

- Phase 0: The main intention of this phase is to obtain fontanelle thickness measurements with a device marketed for this purpose in order to retrospectively improve the measurement algorithm of the device being studied; Neosonics.

Hypothesis 0c: The thickness and attenuation of the fontanelle does not vary significantly in the area of interest.

Before commencing Phase 0-NS, bench testing will be performed using physical models (phantom) with the Neosonics device for users to compare how structures are detected with Neosonics vs. the Toshiba commercial device.

- Phase 0-NS: The objective of this phase is to detect anatomical structures observed in Phase 0, but at high resolution. The images acquired will help improve the segmentation of the images and algorithms used in Phases 1 and 2 of the study, as well as to improve the handling of the equipment.

- Phase 1: The objective of this phase is to demonstrate safety of the device by using a preliminary diagnostic assessment in patients with or without meningitis between Neosonics and lumbar puncture. In addition, the knowledge gained from the use of Neosonics on patients will allow the improvement of, if necessary, the measurement algorithm.
Hypothesis 1a: The higher the cell concentration obtained by CSF analysis, the higher the echo density observed in the CSF ultrasound image.

Hypothesis 1b: The cell concentration obtained by traditional CSF analytical method will be positively correlated with the CSF echo density obtained by high-frequency ultrasound imaging.

- Phase 2: The main intention of this phase is to determine the sensitivity/specificity of the device to identify the degree of diagnostic agreement between Neosonics and the clinical diagnosis, to determine the agreement between measurement methods (cellularity), as well as to assess patient follow-up capabilities by monitoring the cellularity development.

b) Intended applications and performances of the research product to be verified;

As detailed in Phases 1 and 2, the study is intended to verify the sensitivity and specificity of the probe by comparing the results with the LP, as well as the consistency in cell counting between devices.

c) expected risks and adverse effects of the product to be determined.

No risks or adverse effects are expected in the use of this device in the clinical trial.

A.1 Design of the clinical investigation

A.1.1 General points

a) Description of the type of clinical investigation to be conducted (for example, double blind comparative study, parallel design, with or without a control group) with justification for the choice;

- Phase 0: Observational study of the fontanelle using a commercially available Toshiba device.

There is no control group.

Sample size: 40 patients with an open fontanelle

This is a multi-centre study as it is designed between HULP and HUQM.

- Phase 0-NS: Observational study of the transfontanellar space using Neosonics investigation equipment. Prior to acquisition with Neosonics, an acquisition with the standard ultrasound scanner available.

There is no control group.

Sample size: 60 patients with an open fontanelle (40 patients from HULP and HUQM and 20 patients from HSJD)

This is a multi-centre study as it is designed between HULP, HUQM and HSJD.
- Phase 1: Multi-centre crossover clinical study in which each individual consecutively undergoes each of the procedures (Neosonics + LP). In cases where patients with a confirmed diagnosis by LP are recruited, the acquisition will be performed with Neosonics in the shortest possible time.

In addition, prior to acquisition with Neosonics, an acquisition with the standard ultrasound scanner available will be carried out.

The study is expected to be blind, as the user is not aware of the LP results when taking the Neosonics measurement. However, if the recruitment of patients with a negative meningitis diagnosis is very high, patients with a confirmed LP diagnosis can be recruited (cellularity above the diagnostic threshold). In this case, it may be that the investigator who recruited the patient does know the LP results. Recruitment of patients with a confirmed LP diagnosis will reduce the recruitment of patients with a negative meningitis diagnosis, ensuring that the number of patients recruited is limited to that is indicated in the protocol and the ethical criteria of a clinical trial.

Sample size: 16 newborns/infants less than 12 months old or with an open fontanelle and an indication for LP (6 of which have confirmed meningitis with cellularity values above the diagnostic threshold).

This is a multi-centre study as it is designed between HULP, HUQM and HSJD. If, during the analysis of images obtained with Neosonics it is seen that the quality is poor in any of the patients, the patient can be excluded from the analysis and replaced with another to meet the objective of being able to analyse 10 patients with a cellularity below the diagnostic threshold) and 6 with a positive meningitis diagnosis. This is estimated to occur in a maximum of 10 patients, which could mean the final recruitment of 26 patients instead of 16.

- Phase 2: Multi-centre crossover clinical study in which each individual consecutively undergoes each of the procedures (Neosonics + LP). In cases where patients with a confirmed diagnosis by LP are recruited, the acquisition will be performed with Neosonics in the shortest possible time.

In addition, prior to acquisition with Neosonics, an acquisition with the standard ultrasound scanner available will be carried out if considered necessary.

The study is expected to be blind, as the user is not aware of the LP results when taking the Neosonics measurement. However, if after preliminary analysis it is found that the recruitment of patients with a negative meningitis diagnosis is very high, patients with a confirmed diagnosis by LP can be recruited. In this case, the recruitment will not be blind, but the Neosonics acquisition will be blinded to the LP results, and should be carried out as soon as possible once the LP results are available, preferably within less than 24 hrs 8h: Recruitment of patients with a confirmed LP diagnosis will reduce the recruitment of patients with a negative meningitis diagnosis on the basis of CSF cellularity, ensuring that the number of patients recruited is limited to that is indicated in the protocol and the ethical criteria of a clinical trial.

Sample size: 170 newborns/infants less than 12 months or with an open fontanelle and with indication for LP (77 of whom must have meningitis). This is a multi-centre study as it is designed between HULP, HUQM, HSJD and HER.
b) description of the measures to be taken to minimise or avoid bias, including randomisation and blind/blinded procedure;

There is no considered bias in the inclusion of patients, as those who meet the inclusion criteria will be treated in a randomised fashion. However, the measurement of white blood cells in phases 1 and 2 with the research device which is a follow-up procedure in most cases will be blinded for the user, who will not have the LP test result data which is a follow-up procedure to the Neosonics scan in most cases.

Only if the analysis of the preliminary results shows that many patients are being recruited with a negative meningitis diagnosis (cellularity below the diagnostic threshold) and there is a risk that recruitment of patients will be terminated and not enough patients with meningitis will be tested, will patients with a diagnosis confirmed by LP be recruited. In these cases recruitment will not be blind, but the acquisition will be blind with Neosonics and will be performed as soon as possible after the results of the cell count are available (<24hrs 8hrs).

c) Primary and secondary assessment criteria, with justification for the selection and measurement; Phase 0 will be complete once 40 images of the transfontanellar space have been obtained and changes to the Neosonics measurement algorithm can be made in order to begin phase 1.

Phase 0-NS will be complete once images of the transfontanellar space of 40 patients have been obtained, which allow the detection of the anatomical structures and the implementation of the algorithms, and the segmentation of the images.

The completion of the recruitment of the 20 patients in the HSJA (Phase 0-NS) is not a requirement to commence Phase 1.

The device is considered to have passed Phase 1 if the success rate is 95% compared with the LP results, once the measurement algorithm has been fine-tuned.

- Main variables:

  **Sensitivity**: The probability of correctly classifying an individual with the disease, i.e. the probability of a positive test result for a subject with meningitis. Sensitivity is, therefore, the ability of the test to detect the disease, so it can also be defined as the fraction of true positives (FTP).

  **Specificity**: The probability of correctly classifying a healthy individual, i.e. the probability of a negative test result for a subject without meningitis. In other words, specificity can be defined as the ability to detect healthy individuals, and can also be defined as the “fraction of true negatives (FTN)”.

  **Cell count**: Allows detection of the magnitude of cells in the CSF. The count can be performed manually or with semi-automated methods, as set out by the centre.

- Secondary variables: Costs, length of stay, days of treatment.

d) methods and schedule for assessing, recording and analysing the variables;
An intermediate analysis at least will be carried out in all phases to see what trend the results show. In addition, in order to see how the distribution of patients with suspicion is taking place and to analyse the rate of recruitment, several intermediate analyses will be carried out in Phases 1 and 2 with this main objective in mind, and to determine whether it is necessary to recruit any patients with a confirmed diagnosis in order to adjust the recruitment.

e) equipment to be used to determine the clinical investigation variables and plans to monitor the handling and calibration.

- Toshiba, Aplio 400/500, GE Logiq S8 or another with similar features (in all phases) and Neosonics (in Phases 0-NS, 1 and 2)

- Neosonics (except in Phase 2) is connected to a computer or processing module which will collect the scan images.

- The analytical and clinical variables and iconography will be obtained from the patient's clinical history.

- Subsequent image processing will be performed with the digital reconstruction software referred to above.

f) any procedure for subject substitution.

Any patient who declines to participate in the study at any stage will be immediately withdrawn and a new patient will be recruited in their place with the corresponding enrolment code.

If, during the analysis of images obtained with Neosonics in Phase 1 it is seen that the quality is poor in any of the patients, the patient can be excluded from the analysis and replaced with another to meet the objective of being able to analyse 10 patients without meningitis and 16 with meningitis confirmed by LP. This can only be done in Phase 1 (concept test), taking into account the low number of patients included according to the design. This is estimated to occur in a maximum of 4-5 patients, which could mean the final recruitment of 30 patients instead of 26.

All patients undergoing Neosonics acquisition will be covered by the trial's policy.

Reagents

Equipment

Procedure

A.1.1 Research and testing product(s)

a) Description of the exposure to the research and testing product(s), if used;
Exposure to the research product will only occur during the patient screening procedure (Phase 0-NS) and in Phases 1 and 2 with the performance of the LP.

b) justification for the choice of test(s);

There is no control group in the study because all the patients undergo both procedures.

c) List of any other medical device(s) or medications(s) to be used during the clinical investigation; None other than the conventional ones in the routine clinical practice of the patient with suspected meningitis.

d) Number of research devices to be used, together with a justification.

The number of research products is one; the Neosonics described in this application.

However, a consumable is also required for its use, which consists of a membrane that is placed on the tip of the probe to improve transmission of the US and the sensor rotation.

One consumable per patient will be used in Phase 0-NS, and more than one consumable can be used in Phases 1 and 2 to perform follow-up measurements on the same patient and view the efficacy of the treatment in patients diagnosed with meningitis. Scans can be performed every 24 hours in the first three days and every 48 hours until the completion of the treatment or the device does not detect cellularity.

If the consumable breaks it must be replaced with a new one. Regarding the device, one unit will be left in neonatology and it will be replaced in case of breakage or failure.

A.1.2 Subjects

a) inclusion criteria for subject selection;

Phases 0 and 0-NS: Premature newborns, term newborns and/or infants with an open fontanelle and who may or may not have signs of meningitis.

Informed consent by parents/guardians

Phases 1 and 2:

Newborn and infant with an indication for lumbar puncture and with an open fontanelle. Informed consent by parents/guardians

b) exclusion criteria for subject selection; In all phases:

History of cranio-encephalic trauma. Closed fontanelle.

Lack of informed consent by parents/guardians

c) criteria and procedures for the withdrawal or release of subjects;
The participants can leave the study whenever they wish; dropouts will be substituted with new cases so the number of patients is as outlined.

d) timing of inclusion;

Taking into consideration that Phase 0 aims to obtain transfontanellar imaging using a device marketed and approved for this purpose, this phase will begin from the date of the Clinical Research Ethics Committee's (CEIC, abbreviated in Spanish) approval as it does not require express authorisation by the competent authority (Spanish Agency of Medicines and Medical Products) (AEMPS, abbreviated in Spanish).

However, the subsequent phases in which the research equipment will be used must be authorised by the Clinical Research Ethics Committee's (CEIC, abbreviated in Spanish) and the Spanish Agency of Medicines and Medical Products (AEMPS, abbreviated in Spanish). The inclusion of Phase 0-NS patients may be carried out in parallel to the inclusion of patients for Phase 1, as the patient profile is different and the study objective is also different. However, Phase 2 will begin once Phase 1 is completed.

e) total expected duration of the clinical research;

18 months

The duration of the study has been altered due to the emergence of Covid-19, as well as by the availability of medical equipment at certain times.

f) expected time for the participation of each subject;

Scanning times is estimated at 20 minutes per patient with both devices.

g) number of subjects that need to be included in the clinical investigation and estimated time needed to select this number (i.e. inclusion time).

- Phase 0: 40 patients (10 premature, 10 newborns, 10 infants from 1-3 months and 10 infants from 4-12 months or older ones with an open fontanelle). Estimated inclusion time: 2 months

- Phase 0-NS: 60 patients (15 premature newborns, 15 newborns at term, 15 infants from 1-3 months and 15 infants from 4-12 months or older ones with an open fontanelle). Estimated inclusion time: 2 months

- Phase 1: 16 patients (6 of which must be patients with LP-confirmed meningitis). Estimated inclusion time: 3 months

- Phase 2: 170 patients (77 of which must be patients with LP-confirmed meningitis).
Estimated inclusion time: 9 months

**A.1.3 Procedures**

a) description of all procedures related to the clinical investigation that the subjects will undergo during its performance;

- Phases 0 and 0-NS:

  **Clinical procedure**

  Parents/guardians will be offered entry into the study for their child and will be given the information and informed consent document.

  In Phase 0, if the patient's entry into the study is accepted by parents/guardians and after signing the informed consent document a member of the research team will perform the imaging tests with the standard equipment, following the current hospital protocol and carrying out the series of studies instructed by the medical team responsible for the patient.

  In Phase 0-NS, if the patient’s entry into the study is accepted by parents/guardians and after signing the informed consent, document a member of the research team will perform the imaging tests with the standard equipment, and consecutively with the design equipment, through the anterior fontanelle. The conventional neuroimaging procedure will follow the current hospital protocol, performing the series of studies instructed by the medical team responsible for the patient. Regarding the research device, once the device has been positioned according to the established recommendations, 3 consecutive acquisitions will be taken in the same session with both devices.

  **Image acquisition and confidentiality measures (Phase 0-NS)**

  Transfontanellar sonography will be performed using the standard and high frequency ultrasound equipment selected for the study. In both cases, the images will be transferred to a laptop. The examination preparation will not take more than 10 minutes; neither will the scanning time exceed 20 minutes per device. A guiding line will be used (rectangular digital line or frame superimposed on the ultrasound image to guide the operator’s positioning). When the frame is completely over the space where the CSF is located, the image is ready to be transferred and stored on the disk. Three acquisitions will be performed with the frame inside the CSF for further measurement accuracy studies.

  A new file will be created for each patient which will not contain any personal information in accordance with the Law of Confidentiality, in compliance with Spanish legislation and the new European Regulation 2016/679 of the European Parliament and of the Council, dated 27 April 2016. The name of the file will be a code so that it is not possible to link the information to the patient. The link
between the patient's personal and clinical data and the measurements obtained from the ultrasound data and any images of interest will be kept in a password-protected held by the principal investigator,

_Ultrasound data processing_

Structural and geometric measurements: The different layers that make up the fontanelle (skin, dura mater, arachnoid, CSF, pia mater) will be classified by an imaging expert. Then, the images will be used to take manual measurements of the layers using visualisation software (for example, *ImageJ*). The thickness of the different layers at the tissue interfaces is defined by an orthogonal digital line. This information is used to calculate the tissue attenuation.

Physiological measurements: The raw data from the acquisition with the ultrasound system will be used to define the acoustic properties of the segmented layers. First, the echoes at the interfaces between layers will be identified. Second, the range of these echoes will be used to calculate the acoustic impedance and attenuation presented by each layer following the Baddour and Kolios models [10]. After this processing, differences in the echogenicity of the layers between subjects will be assessed.

_Ultrasound system parameters_

The gain parameters of the ultrasound system will be adjusted to obtain an optimal signal from the CSF. These parameters are saved together with the image data and may be reviewed for subsequent data analysis. Regardless of the settings established, the ultrasound system is limited in power output so that it will always comply with the safety limit regulations set by the EC and FDA for transfontanellar ultrasonography (94 mW/cm²).

- Phases 1 and 2:

_Clinical procedure_

Parents/guardians will be offered the opportunity to enter their child into the study and will be given the information and informed consent document, although this must not delay the performance of the lumbar puncture for the meningitis screening, which has been decided by the doctor in charge of the patient, as the CSF is immediately sent to the central laboratory for analysis. Nor must the performance of neuroimaging studies (conventional ultrasound, MRI) be delayed, if they are indicated.

The lumbar puncture procedure will follow the current hospital protocol. The CSF analysis will be carried out in the Emergency Laboratory (Central Services), in accordance with the hospital's standard practice. The CSF cell count procedure is performed by optical microscopy using the Fuchs-Rosenthal chamber. The analytical objective of the laboratory is a variable interobserver of 5% and interobserver of 15% and 5%, for low cell count and (>30 cells/μL) for high, respectively.

The conventional neuroimaging procedure will follow the current hospital protocol, performing the series of studies instructed by the medical team responsible for the patient.
If the patient’s entry into the study is accepted by parents/guardians and after signing the informed consent document, a member of the research team will perform the imaging tests with the design equipment through the anterior fontanelle. 3 consecutive acquisitions will be taken in the same session. The operators, both in the laboratory and in the acquisition of images, will remain blinded to the result of the complementary test, i.e. image and CSF cell count by the laboratory, respectively. The results obtained with both methods will be compared. CSF samples contaminated with blood or in which the white blood cell count cannot be corrected will be discarded.

In the event that after preliminary analysis of the results it is identified that the number of patients with a negative meningitis diagnosis is much higher than the number of diagnosed patients, recruitment of patients with a confirmed diagnosis by LP may be performed. In this case, once the results of the LP are known, the patient can be recruited for the study and once the signed informed consent document has been obtained, the imaging test will be performed with Neosonics as described above, in the shortest possible time.

**Image acquisition and confidentiality measures**

Transfontanellar ultrasonography will be performed using the high-frequency ultrasound equipment selected for the study and the images will be transferred to a laptop computer. The examination preparation does not take more than 10 minutes; neither will the scanning time exceed 10-20 minutes. In Phase 1, with the assistance of the computer and special software, the user can view the image obtained and is guided where to position the probe on the measurement area. Image acquisition at this stage is done in the research phase and once the strobe has been placed on the measurement area. The images will be stored in the equipment and later transferred for analysis. In Phase 2, when the system is automated, the algorithm will calculate the cell concentration in the selected CSF space and a message will trigger the mechanism indicating that the measurement is complete.

A new file will be created for each patient which will not contain any personal information in accordance with the Law of Confidentiality, in compliance with Spanish legislation and the new European Regulation 2016/679 of the European Parliament and of the Council, dated 27 April 2016. The name of the file will be a code so that it is not possible to link the information to the patient. The link between the patient’s personal and clinical data and the measurements obtained from the ultrasound data and any images of interest will be kept in a password-protected held by the principal investigator.

**Semi-automatic cell counting algorithm**

The images obtained will be used to perform a manual and automatic count (see section II. Hypothesis 1.) using an algorithm developed by the group and based on cell tracking algorithms found in optical microscopy imaging tools. The manual count will assess the efficiency of the automatic method in detecting cells in the image.

Prior to the execution of the algorithm, Machine Learning algorithms will be used to segment the tissues of the fontanelle automatically and label the tissues in order to identify the CSF. These algorithms would be
defined and tested with the images acquired in Phase 0-NS.

b) description of the activities carried out by the sponsor’s representatives (excluding the testing);

N/A

c) any known or predicted factors that may compromise the outcome of the clinical investigation or the interpretation of the results.

Factors include the characteristics of the subject during the qualification period, concomitant medication, the use of other medical devices, and subject-related factors such as age, gender, or background, and will be described in the DCB.

The use of Neosonics in the clinical trial does not involve any change in the patient’s treatment or medical care that they may receive, as the management will depend solely on the results obtained from the LP, in accordance with standard clinical practice.

Likewise, the date and time of the LP, as well as the date and time of the analysis of the CSF sample and the time of acquisition with the research equipment will be recorded in the DCB, so that possible differences can be analysed and traced.

A.1.1 Monitoring plan

The Monitoring Plan is intended to detail, subject to acceptance by the study sponsor, the scope and nature of the monitoring procedure appropriate to the clinical investigation.

To ensure proper monitoring of the study, the monitor will conduct an initial visit to the Spanish centres, Mozambique and Rabat. In terms of monitoring, two follow-up visits will be made (face-to-face or by telephone as appropriate), during which the following will be checked:
a) the maintenance of and compliance with the CIP as well as international standards and regulatory requirements

b) that only authorised people are taking part in the clinical investigation,

c) Neosonics is being used in accordance with the CIP or the instructions for use. In the event that amendments to the product, method of use or CIP are required, it will be checked that the sponsor is notified;

d) the research site resources, the availability of investigators, and that the diagnostic methods say correct during the trial;

e) the principal investigator continues to have access to an adequate number of research subjects and products;

f) signed and dated informed consent documents have been obtained for each subject at the time of inclusion or prior to any procedures related to the clinical investigation being undertaken, except where urgent treatment is required (see point 13).

g) source documents and other records of the clinical investigation are accurate, complete, up to date, and appropriately stored and maintained;

h) the DCBs have been registered on time and are consistent with the source documents;

i) appropriate corrections, additions or deletions have been made to the DCBs, including the date and explanation if necessary, and that they show the initials of the principal investigator or authorised designee; the monitor should not make any corrections, additions, or deletions in the DCB.

j) all adverse events and product deficiencies are reported to the sponsor, and all serious adverse events and product deficiencies that could have left serious adverse product effects are reported to the sponsor without undue delay;

k) research products are properly stored and the traceability procedure is followed;

l) all other reports, notifications, applications, submissions and correspondence are maintained in the investigator’s files and are accurate, complete, submitted on time, legible, dated, and identify the clinical investigation;

m) current laboratory standards, and sterilisation service authentications are current and up to date.

n) subject withdrawals are documented; the monitor should discuss this with the principal investigator or authorised designee;

o) non-compliance of the subject with the requirements detailed in the informed consent is documented; the monitor should discuss this with the principal investigator or authorised designee;
the principal investigator and the research team are informed and aware of all relevant documentary updates relating to the clinical investigation;

- In the event that a serious adverse incident is detected, the monitor will be responsible for communicating with the Spanish Medicines Agency to report the event. In addition, they will work with the research team to find the cause of the event and to minimise the risk introducing regulatory measures if necessary.

In addition to the follow-up visits, a monitoring report will be produced after each phase which will should detail:

- date, centre identification, name of the monitor and name of the principal investigator or other individuals contacted, and

- a summary of the aspects reviewed and their observation(s) as to whether outstanding prior actions were completed, as well as significant findings, facts, deviations, conclusions and recommended actions to ensure compliance.

A copy of this report will be given to the principal investigator.

In order to ensure the monitoring and control of all these points, the monitor will have access to a computer system that collects all the relevant patient information (diagnostic tests, courses, documentation, reports, informed consent documents etc.), as well as to the database.

The monitor will confirm their ethical use of this database, to only consult the data relating to the clinical trial in progress.

In addition, the monitor will have access to all documentation relevant to the clinical trial:

- Clinical investigation plan (CIP),
- Investigator’s Manual (IM)
- Patient’s informed consent document and patient information form (IC)
- Data collection books (DCB)
- Instructions for use (IFU)
- Clinical trial safety policy
- and any other written agreement on the clinical investigation (CI)
A.2 Statistical considerations

With regard to chapters A.5 and A.6, the description of and justification for:

a) the design, method and statistical analysis procedures;

For Phase 0, the different soft tissue layers above and below the fontanelle should be identified and labelled. Together with the echo amplitude data of the tissue interfaces and their thickness, the attenuation of each tissue will be calculated. From the pial layer, a region will be chosen where there is orthogonality between the tissue and an imaginary line connecting to the centre of the transducer. This region will always be of the same size and the average of the intensity values will be calculated. From the thickness and attenuation measurements, averages and variances will be calculated as preliminary descriptors of the variability of the measurements. In conjunction with these descriptors, statistical methods allowing intra- and inter-group comparison may be used, and data will be expressed as mean ± SD.

For Phase 0-NS, the analytical method and procedure described in Phase 0 will be followed.

For Phases 1 and 2 and in order to compare the meningitis detection capability between Neosonics and the standard diagnosis ROC (Receiver Operating Characteristic) curves will be used which show sensitivity as a function of false positives (complementary to specificity) for different cut-off points. In addition, paired-data tests (Mc Nemar) will be used to buy cell numbers between techniques and linear regression analysis will possibly be used to assess treatment efficacy by repeated non-invasive measurements.

With the specific objective (sensitivity to cellularity in CSF) in Phase 2 the association of measurements between the two methods will be represented in a scatter plot (see Hypothesis 2b). To assess the differences between measurements the Bland&Altman analysis will be used, in which the bias between the methods and the variability between pairs of measurements will be observed. The sample size is based on the fact that 95% of the measurements are within clinically acceptable limits of agreement (see A7.b). The average difference between methods will be analysed using either the t-student for paired data or the Wilcoxon signed-rank test, depending on whether the data follow a normal distribution or not (and maintain symmetry), respectively.

The CSF cell concentration will be obtained from the cell count provided by the hospital's analysis department and will constitute the reference. To obtain a measurement of the ultrasound data concentration, 660 sequential acquisitions will be taken in 10 seconds, equivalent to 220 lines per 3 measurements. To achieve 95% confidence in the detection of the cell concentration with an error of 3 cells/μL (d) with respect to the actual concentration and with a measurement variability between acquisitions of 10 cells/μL (σ), the total number of cells (N) to be detected is 96 (N = σ²Z²).
Based on \textit{in vitro} experiments this number is easily achievable even at concentrations below 5 cells/μL.

b) the sample size

There are different methods to estimate the sample size of the study depending on the objective: (1) Diagnostic sensitivity and specificity of the equipment (primary objective) or (2) concordance in CSF cell count (secondary objective).

1) According to FDA guidelines [11, 12] and previous literature [13, 14], the sample size in Phase II has been calculated taking into account that Neosonics has a sensitivity of 95% (FN of 5%; TP of 95%), a specificity of 80% (TN of 80%; FP of 20%) compared to lumbar puncture. With these values and having a 95%CI (94.73%-101.27%), a sample size of 77 patients with meningitis (confirmed by LP, cellularity above the diagnostic threshold) and 77 without meningitis is estimated. Taking into account 10% deviations (technical, protocol, or loss of follow-up), the final sample size with suspected meningitis should be \( \sim 170 \) patients.

2) In line with the second objective, Phase II is designed as a technological feasibility trial to determine the ability of Neosonics to count cellularity in CSF compared to manual or haemocytometer cell counting. In this context, one of the main variables to be taken into account is the cell concentration (cell/μL); a variable with which will be compared with the differences in cell count between the agreed methods of Bland & Altman [Bland & Altman]. For this purpose, taking into account the diagnostic criterion of meningitis (20 cells/μL and 9 cells/μL for newborns and children aged 29-90 days, respectively [39-41]), a degree of agreement between methods of ±3 cells/μL has been considered acceptable. Therefore, it is essential that the CI (95%) of the counting differences do not exceed these limits of agreement. The CI depends on the variability between methods and is obtained from the calculated standard deviation between the two measurement methods (haemocytometer counting or manual counting). Given the variability observed in the preliminary data, the standard deviation difference is equal to 1.24 cells/μL. Therefore, with a target recruitment of 154 patients (considering the final sample size without deviations) the 95%CI of the difference between the cellularity measurements, lies between 2.09 to 2.77 cells/μL above the defined limit of agreement. If 10% deviations are added to this sample size (as in the previous sample size estimation), a final sample size of 170 patients is estimated.
Therefore, a sample size of ~170 patients will provide sufficient evidence to answer both objectives: (1) sensitivity and specificity of the device vs. LP and (2) concordance in terms of cell count.

Considering that the incidence of patients with meningitis is low in the European population and a distribution of 50% cannot be estimated, to ensure the recruitment of 77 patients with a diagnosis of meningitis, it is proposed to recruit patients with a confirmed diagnosis of meningitis by LP if preliminary analysis of the results suggests this.

c) the level of significance and statistical strength of the clinical investigation;

A value of p=0.05 will be considered as statistical value and a strength of 80%.

d) the expected dropout rates;

1%

e) the acceptance/rejection criteria to be applied to the results of the clinical investigation;

The study has been designed to demonstrate that the device is a suitable diagnostic tool, aiming for a sensitivity of 95% (FN: 5%, TP: 95%) and a specificity of 80% (TN: 80%, FP: 20%). However, market surveys of opinion leaders place the average expected sensitivity at around 91% (with a range between 80-99%), which is lower than the value used to calculate the sample size.

If attention focusses on the second objective of the study, the current design also allows the assessment of the concordance between the results of the cell count between the LP and Neosonics (estimated to be 3 cells/μL), however, the impact of this error in the cell count will be different if we are far from or close to the diagnostic threshold.

Therefore, if the results obtained at the end of the test are below these values in terms of sensitivity but the concordance of the results between cell counts is acceptable from the point of view of the diagnostic threshold for meningitis and is similar to the current method, the equipment is considered to be a suitable diagnostic tool. Therefore, the need for re-analysis of the acquisitions obtained with the equipment will be assessed to improve the counting algorithms or to replicate the study with a larger sample size if necessary.

f) The provision of an interim analysis, where appropriate;
As discussed above, in Phases 1 and 2 it is envisaged that at least one interim analysis of the results will be carried out, to assess the quality of the results obtained and the rate and distribution of the patients recruited.

g) criteria for discontinuation of the clinical investigation based on statistical results.

Not applicable.

h) procedures for reporting any deviation from the original statistical plan; Any deviation from the original investigation plan will be communicated to the Spanish Agency of Medicines and Medical Products (AEMPS, abbreviated in Spanish);

Any observed deviations will be recorded in the Investigation Database Deviation Protocol (IPDD-01PIC-UNITED) in accordance with the NBS-IDP.

i) subgroup specification for the analysis;

The subgroups considered are defined in the study design.

j) the procedures that take into account all the data;

All variables collected in the study will be stored in a prospective collection database.

k) the processing of missing, unused or spurious data, including dropouts and withdrawals;

In Phase 1, patients with poor quality images may be replaced by others. A double analysis will be performed considering patients with any relevant missing variable and excluding any patient with any relevant missing variable.

l) exclusion of information specific to the hypothesis trial, if relevant,

N/A
m) in multicentre clinical investigations, the minimum and maximum number of subjects to be included at each centre.

Special rationale and sample size(s) may be applicable for early stage clinical investigation(s), for example, feasibility clinical investigation(s).

In Phase 0-NS, recruitment is expected to be 40 patients from HULP and HUQM and 20 patients from HSJD.

In Phase 1 patients will be included from the HULP, HUQM and HSJD centres. Recruitment of 8 patients from HSJD (5 negative and 3 positive patients) is expected. The remaining patients will be recruited mainly from HULP. The recruitment ratio from HUQM is expected to be lower.

The financial support for these two centres has been approved by the medical team.

Regarding Phase 2, taking into account that the incidence of meningitis is similar to the European level, although it may be slightly higher in Morocco, it is expected that up to 60 patients could be recruited from the state centres (HULP, HUQM and HSJD).

A.3 Data management

The IP of each centre will review the data collection booklet prospectively to ensure that the information is complete and valid, as well as to check adherence to the protocol. Only the IP of each centre and the monitor will have access to the patient’s demographic or other personal information, which will be stored on a password-protected server (FTP server_ File Zilla).

The data obtained from the ultrasound equipment will be anonymised immediately at the time of collection according to the new European data protection regulation (EU 2016/679). A numerical code will be applied to name the files and anonymise data. The files will be transferred from the ultrasound equipment to an external disk and shared with NBS via password-protected encrypted protocols for subsequent data processing. The original informed consent documents will be stored under lock and key at each institution and/or digitised and uploaded to the patient’s medical records. Electronic data collection from the participants will also be encrypted.

A weekly back-up of the database containing all the parameters described in the data collection notebook will take place. A copy of the prospective collection database will be kept for up to 10 5 years
after the end of the study and will be treated with the same degree of confidentiality as the rest of the data in the patients' medical records.

A.4 Amendments to the CIP

The IB, CIP, CRF, informed consent document, and other subject information, or other clinical investigation documents will be amended as necessary during the clinical investigation including a by including a justification statement with each amended section of a document. Proposed amendments to the CIP should be agreed between the sponsor and the principal investigator. Amendments to the CIP and to the subject’s informed consent document should be reported to, or approved by, the EC and regulatory authorities, if required. The changes, version number and date of the amendments will be documented in accordance with UNE-EN ISO 14155:2012

A.5 Deviations from the clinical investigation plan

The investigator will not deviate from the CIP except:

- Upon justified request and approval by the Ethics Committee.

- In emergency circumstances, deviations from the CIP to protect the rights, safety, and welfare of human subjects may take place without prior approval by the sponsor and the Clinical Research Ethics Committee (CEIC, abbreviated in Spanish). Such deviations should be documented and reported to the Clinical Research Ethics Committee (CEIC, abbreviated in Spanish) and the Spanish Agency of Medicines and Medical Products (AEMPS, abbreviated in Spanish) as soon as possible.

NOTE: For non-substantive changes [e.g., minor logistical or administrative changes, change of phone numbers, renewal of insurance policy] that do not affect the rights, safety, and welfare of the human subjects and are not related to the objectives or assessment criterion of the clinical investigation, a single notification to the Clinical Research Ethics Committee (CEIC, abbreviated in Spanish) and, where appropriate, the regulatory authorities may be sufficient.

The requirements and procedures for recording, reporting and analysing deviations from the CIP will be in accordance with UNE EN ISO/IEC 17025-1 and performed as soon as possible.
A.6 Product recording

Access to research products will be controlled and will only be used in clinical research and in accordance with the CIP.

The manufacturer will maintain records to document the physical location of all research products from shipment to the research centres until their return or disposal.

The principal investigator or an authorised designee will maintain the records documenting receipt, use, return, and disposal of the research products. The records will include:

a) date of receipt;

b) identification of each research product (batch number/unique serial code number);

c) expiry date, if applicable;

d) date(s) of use;

e) date on which the research product was withdrawn, if applicable;

f) the return date of research products that have not been used, have expired or are faulty, if applicable.

Meanwhile, NBS as manufacturer will store the documentation of all devices and consumables supplied to the centres and related to the customer relationship (delivery notes, returns, ...) as well as any documentation associated with their manufacture.

A.7 Compliance declarations

The Declaration of Compliance is attached to the documentation to be submitted to the Spanish AEMPS, in which the following requirements are detailed:

a) that the clinical investigation must be carried out in accordance with the ethical principles to be complied with in the Declaration of Helsinki

b) international standards and any regional or national regulations, as appropriate;

c) that clinical investigation using the device for the study will not commence until approval/a favourable decision has been obtained from the AEMPS;

d) That any additional requirement of the Clinical Research Ethics Committee (CEIC, abbreviated in Spanish) or the AEMPS has been met;
e) The CI subjects are entitled to insurance, as indicated in the informed consent document

A.8 Informed consent procedure

Patients who are candidates for the study may come from the Emergency Department or from the paediatric and neonatal hospitalisation units of these centres.

The Principal Investigator as well as the doctors participating in the study, will be responsible for informing the patient’s parents or guardians about the inclusion in the clinical study during the consultation. The doctor will inform the family about the potential benefits and risks of the device and will resolve any of the patient’s doubts. This will be recorded in the patient’s records. Upon approval by the patient and at the request of the family, the doctor will provide the patient with a copy of the informed consent document.

Once all doubts have been resolved, the informed consent document will be signed by the person in charge and the researcher.

- When it is not possible to obtain the subject’s prior informed consent, and their legally authorised representative is not available, the subject may still be included in the study if their treatment is clearly likely to improve their health. In this case, the subject or the subject’s legally authorised representative should be informed as soon as possible; by supplying them with all the required information.

This document will be incorporated into the hospital’s documentation system to constitute its viability.

Appendices 8-10 contain the current revisions of the information and informed consent pages for each phase of the study.

A.9 Adverse events, serious adverse events and product deficiencies

a) definitions of adverse events, serious adverse events and product deficiencies:
Section A.14 follows that described in MDCG 2020-10/1 entitled “Safety reporting in clinical investigations of medical devices under the Regulation (EU) 2017/745”.

- Adverse event (AA): Any untoward medical incident, unanticipated illness or injury, or adverse clinical sign, including an abnormal analytical result, that occurs in subjects, users or other people within a clinical investigation, whether or not related to the research device;

- Serious adverse event (SAE): All adverse events that has any of the following results:
  a) death,
  b) serious deterioration in the subject’s health resulting in: (i) life-threatening illness or injury, (ii) permanent deterioration of the subject’s health, of a bodily structure, (iii) hospitalisation or prolonged hospitalisation of the patient, (iv) medical or surgical intervention to prevent a potentially life-threatening illness or injury or permanent deterioration of a bodily function or bodily structure, (v) chronic illness, c) foetal distress, stillbirth or a physical or mental impairment or congenital malformation;

- Product deficiencies; any inadequacy in the identity, quality, durability, reliability, safety or efficacy of a research product, including malfunction, user error or a lack of information provided by the manufacturer;

a) Procedure for notification of events

By the sponsor to the Competent Authority:

Currently, due to the transition period to the new regulation and the inactivity of some Eudamed modules, the AE notification procedure may vary depending on development status of the platforms.

In accordance with the MDCG 2020-10, the following table summarises the formats to be used for the communication of AEs

By the investigator to the Sponsor:
The investigator should use the NBS-generated event reporting format, which collects critical data which collects the critical data needed for future reporting.

**a) Reportable events**

Once all the information has been collected, in accordance with Art. 80 of the MDR, the investigator should report the event if:

a) it is an adverse event which may have a possible causal effect on the research device, or the clinical investigation plan

b) any device deficiency that could have resulted in an adverse event/serious adverse event.

If appropriate action not been taken, or circumstances had been less fortunate;

a) c) any new findings in relation to any event referred to in points a) and b).

**b) the time period within which the principal investigator is required to report all adverse events and product deficiencies to the sponsor and, where appropriate, to the EC and the regulatory authority;**

**Reporting period following identification of an incident by the sponsor to the Competent Authority:**

- When the event signals an imminent risk of death, serious injury or serious illness and requires prompt corrective action for other patients/subjects, users or other persons or a new finding: **Immediately, but no later than 2 calendar days after the sponsor becomes aware of a new reportable event or new information regarding a new event already reported.**

This includes events that are of a significant and unexpected nature, so as to become a potential public health hazard of concern. This also includes the possibility that multiple deaths may occur in short intervals.

These concerns may be identified by the Competent Authority or the manufacturer.

- Any other reportable event as described in section A.14.a or a new finding/update on the event: Immediately, but no later than 7 days after the date the sponsor is made aware of the new reportable event or new information in relation to one already reported.

**Reporting period following identification of an incident by the investigator to the Sponsor:**

In the case of an event, the investigator should send a report of what happened using the NBS incident report. The time frame for reporting should be as soon as possible, and no later than 3 days after the research centre study personnel become aware of the event.

**c) Causality assessment**

The relationship between the use of the medical device (including the medical-surgical procedure) and the occurrence of each adverse event will be assessed and categorised.
During the causality assessment exercise, clinical judgement will be used and relevant documents, such as the investigator’s file, the Clinical Investigation Plan, or the Risk Analysis in which the expected risks are identified should be consulted.

The presence of confounding factors, such as concomitant medication/treatment, the patient’s medical history, or other diseases should also be considered.

These considerations will be taken into account in the analysis of serious and non-serious adverse events.

For the purpose of consistent reporting each AE will be classified according to four different levels of causality:

1. Unrelated
2. Possible
3. Probable
4. Causal relationship

These 4 situations are described in the MDGC 2020-10-01.

During the investigation, the sponsor together with the investigator should identify which event is related to the product itself and not to other events or situations.

**d)** List of anticipated adverse events and expected adverse effects of the product, together with their incidence, mitigation or possible handling;

No potential adverse effects have been identified in the use of the device.

**e)** Emergency contact information for reporting serious adverse events and product serious adverse effects;

The details for AE reporting to the Competent Authority, until Eudamed is fully operational are below.

**Troubleshooting**

**Time Taken**

**Anticipated Results**

**References**


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No patient will receive any financial benefit for their participation in the study.