BMJ Open

Contribution of contrast enhanced ultrasound in the diagnosis of adnexal torsion (AGATA): protocol for a prospective comparative study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-073301
Article Type:	Protocol
Date Submitted by the Author:	01-Mar-2023
Complete List of Authors:	Pillot, Rémi; CHRU de Nancy Gynécologie obstétrique & néonatologie HOSSU, Gabriela; CHU Nancy, INSERM U1254; CHU Nancy, CIC-IT 1433 CHERIFI, Aboubaker; CHU Nancy, INSERM CIC-IT 1433 Guillez, Kévin; CHRU de Nancy Gynécologie obstétrique & néonatologie, Morel, Olivier; CHRU de Nancy Gynécologie obstétrique & néonatologie Beaumont, Marine; CHU Nancy, Inserm, CIC Fijean, Anne-Laure; CHRU de Nancy Gynécologie obstétrique & néonatologie, Bertholdt, Charline; Obstetric and fetal medicine Unit; IADI
Keywords:	GYNAECOLOGY, Genitourinary imaging < RADIOLOGY & IMAGING, Ultrasound < RADIOLOGY & IMAGING

Contribution of contrast enhanced ultrasound in the diagnosis of adnexal torsion (AGATA): protocol for a prospective comparative study.

R. Pillot[1], G. Hossu[2,3], A. Cherifi[3], K. Guillez[1], O. Morel[1,2], M. Beaumont[2,3], AL. Fijean[1], C. Bertholdt[1,2]

[1] University of Lorraine, CHRU NANCY, Obstetrics and Gynecology Department, F-54000 NANCY, France
[2] University of Lorraine, INSERM U1254, IADI, F-54000 NANCY, France
[3] CHRU Nancy, Inserm, University of Lorraine, CIC, Innovation Technologique, F-54000 NANCY, France

Corresponding author: AL.Fijean
Obstetric and fetal medicine Unit
Maternité Régionale Universitaire, CHRU de Nancy
10, avenue du Docteur Heydenreich
54000 NANCY, France
Email: a.fijean@chru-nancy.fr

ABSTRACT

Introduction: Adnexal torsion is a surgical emergency, the prognosis of which depends on the time elapsed prior to treatment. Sensitivity of pelvic ultrasound remains low and does not allow a clear diagnosis.
The primary objective is to evaluate the diagnostic performance of contrast enhanced ultrasound for the diagnosis of adnexal torsion in women with suspected adnexal torsion. The secondary objectives are: (1) to describe the perfusion parameters of the ovaries by contrast enhanced ultrasound, (2) to compare performance diagnosis of contrast ultrasound with bidimensional Doppler for the detection of adnexal torsion, (3) to describe the perfusion parameters of the ovarian as a function of the degree of adnexal torsion, (4) to compare perfusion parameters before and after ovarian detorsion and (5) to describe perfusion parameters of the ovarian by using MicroVascular Flow technique.

Methods and analysis: This is a monocentric, prospective comparative, non randomised, open and interventional study. We hypothesize to include 30 women: 20 positives cases compared to 10 control cases. Women will be informed and recruited in the emergencies, over a period of 36 months.
The primary endpoint is the signal intensity measurement to assess sensitivity, specificity, positive and negative predictive value of contrast enhanced ultrasound for detection of adnexal torsion in women with suspected adnexal torsion. The presence or absence of adnexal torsion is confirmed during the surgical intervention.

Ethics and dissemination: The study was approved by the French Ethics Committee, the CPP (Comité de Protection des Personnes) OUEST I on July 3rd, 2020 with reference number 2020T1-16. The results of this study will be published in a peer-reviewed journal and will be presented at relevant conferences.

Registration details: ClinicalTrials.gov registry (NCT04522219); EudraCT registry (2020-000993-27).

STRENGTHS AND LIMITATIONS

- This is the first study in adults evaluating the interest of contrast enhanced ultrasound for the diagnosis of adnexal torsion
- This imaging technique has clinical applicability, including in the emergency setting
- This study includes simultaneous evaluation of a functional imaging technique with and without contrast injection
- This study will not be able to assess the potential benefit in the event of false negative, without surgical management
- The sample of patients is small

INTRODUCTION

Adnexal torsion is surgical emergency due to the total or partial rotation of the adnexa as well as the ovary and in rare cases the fallopian tube, around its vascular axis. Most of cases occur during the period of genital activity and the delay in treatment can lead to ischemia of the adnexa (1). This ischemia may lead to hemorrhagic necrosis of the ovary that could impair its functionality and consequently fertility (2). Treatment is exclusively surgical by laparoscopic detorsion and must be performed as soon as possible to preserve ovarian function (3).

Early diagnosis is an essential prerequisite to reduce the potential consequences of ovarian torsion. The short timeframe between the occurrence of torsion and the surgical intervention allows conservative treatment without functional consequences in 90% of the cases (2). Usually, this timeframe is approximately 6 hours.

The clinical diagnosis is challenging since the main clinical sign is pelvic pain of sudden onset, a non-specific sign that does not allow for a precise diagnosis.

To confirm the diagnosis, the gold standard is pelvic ultrasound with Doppler flow analysis (4). However, its contribution is low, with a sensitivity ranging from 46 to 73% depending on the study (5). Other imaging techniques have been considered, such as MRI, with much greater sensitivity than ultrasound, but its difficult accessibility, particularly in the context of emergency, makes it difficult to use in clinical practice (6,7).

Ultrasound therefore remains the most appropriate modality. SonoVue® ultrasound contrast injection improves blood echogenicity and signal-to-noise ratio (8). This product consists of sulfur hexafluoride microbubbles acting as reflectors of the ultrasound beam with a diameter comparable to that of a red blood cell (approximately 6 µm), which makes it a strict intravascular contrast product.

This technique therefore seems perfectly suited to assess the vascularization of the ovary and improve the diagnostic sensitivity of adnexal torsion. Its benefit has already been demonstrated in the diagnosis of testicular torsion in animals, but to date, only one study has evaluated its contribution in adnexal torsion (8,9).

Indeed, the only study analysing the diagnostic performance of contrast enhanced ultrasound in cases of adnexal torsion, is the retrospective study by Trinci et al, including twenty patients from a paediatric population. The sensitivity is 94.1% and the specificity is 100%, i.e. an overall accuracy of 95% (9). It confirms the diagnostic contribution of contrast enhanced ultrasound as a complement to conventional 2D ultrasound in the diagnostic of adnexal torsion.

METHODS AND ANALYSIS

Objectives

The **primary objective** is to evaluate the diagnostic performance of contrast enhanced ultrasound for the diagnosis of adnexal torsion in women with suspected adnexal torsion.

The **secondary objectives** are:

1. To describe the perfusion parameters of the ovaries by contrast enhanced ultrasound.
2. To compare performance diagnosis of contrast ultrasound with bidimensional Doppler for the detection of adnexal torsion.
3. To describe the perfusion parameters of the ovarian as a function of the degree of adnexal torsion.
4. To compare perfusion parameters before and after ovarian detorsion.
5. To describe perfusion parameters of the ovarian by using MicroVascular Flow technique.

Trial design

The AGATA protocol is a monocentric, prospective comparative, non randomised, open and interventional study.

Study population

Women will be informed and recruited in the emergencies, over a period of 36 months. All the women suspected of adnexal torsion and with planned surgery who agree to participate in the study will be recruited, despite their medical history and previous examination results. The inclusion and exclusion criteria are reported in Table 1. The consent collection will be provided by the investigators (obstetricians) (Annex 1).

Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Woman over 18 years old • Woman affiliated to a social security • Woman having received complete information on the organization of the research and having given her informed consent in written form. • Planned surgical intervention for suspected adnexal torsion	• Patients under a measure of legal protection, • Contraindication to contrast injection: Hypersensitivity to sulfur hexafluoride or any of the other ingredients, history of cardiac disease, respiratory distress syndrome, severe pulmonary hypertension.

Ultrasound acquisition

Ultrasound acquisitions take place in three stages:

- Identification of ovaries in two-dimensional ultrasound
- Ultrasound acquisitions without injection of contrast product (Microvascular Flow)
- Ultrasound acquisitions with injection of contrast product (Contrast enhanced ultrasound).

These acquisitions will take place at different times of the medical care:

- Before surgery: bilateral acquisition
- After surgery: unilateral acquisition on the affected ovary in case of confirmed torsion.

The flow chart of women participation is in Figure 1.

The ultrasound acquisition will be performed with a HERA W10 (Samsung) and an endovaginal transducer (3-10 MHz). The ultrasound contrast product used will be Sonovue® (Bracco Imaging, Italy), administered by bolus injection. A volume of 2.4 mL of contrast product will be injected per acquisition with a maximum of 3 injections per women.
The contrast enhanced acquisition will be performed in contrast mode with standardized parameters and the Microvascular Flow acquisition will be performed in MvFlow Mode. Acquisition will be recorded as videoclip for 2 minutes.

Image analysis

The analysis of contrast enhanced ultrasound will be performed with a specific off-line software (Vuebox®, 7.0.26 version, Bracco Suisse SA, Geneva, Switzerland). Region of interest will be manually drawn on the overall ovaries allowing to obtain time intensity curve for the quantitative analysis (semiquantitative perfusion indicators).
The analysis of Microvascular Flow ultrasound will be performed on the sonographer with a specific integrated software. The region of interest will be drawn, and quantitative parameters will be extract (Figure 2).

Outcomes

The **primary endpoint** is the signal intensity measurement to assess sensitivity, specificity, positive and negative predictive value of contrast enhanced ultrasound for detection of adnexal torsion in women with suspected adnexal torsion with realization of ROC curves (Receiver Operating Characteristics).
The presence or absence of adnexal torsion is confirmed during the surgical intervention.

The **secondary endpoints** are:

1. Measurement of perfusion parameters of the suspected ovarian torsion and the contralateral ovary if available: signal intensity and perfusion kinetics.
2. Measurement of signal intensities to assess sensibility and specificity of contrast enhanced ultrasound and bidimensional (2D) Doppler.
3. Comparison of perfusion parameters of the ovary with the degree of torsion. The degree of torsion is defined by the number of twists (number of turns around the axis) detected during the surgical procedure.
4. Measurement of signal intensities before and after ovarian detorsion.
5. Measurement of signal intensities obtained by Micro Vascular Flow technique.

Participant timeline

The enrolment of women is started in April 2021. The recruitment should be achieved by April 2024. The flow chart of women participation is presented in Figure 1.

Premature ending of patient participation

Every person can stop participating in the research at any time and for whatever reason.
The investigator may temporarily or permanently interrupt a person's participation in the research for any reason that has an impact on her safety or that would best serve the interests of the person who is suitable for research.
In the event of a premature ending or in the event of the withdrawal of consent, the withdrawal shall not affect the activities carried out and the use of data obtained on the basis of informed consent before it has been withdrawn, unless the person indicates in writing that she objects to their use.
When a patient withdraws her consent, the data acquired before the withdrawal will be available for the statistical analysis unless refusal from the patient.

Follow-up

A clinical examination will be performed on the inpatient ward at 24 hours by an obstetric gynecologist to look for any adverse events that have occurred since the inclusion visit.

Sample size consideration

The innovative nature of this research does not allow us to have published figures on which we can rely to estimate a number of women to be included. We choose to include 30 women. With an assumed distribution of 20 positives cases against 10 control cases, an accuracy of 5%, a power of 95%; this sample will give an area under the curve of 85% (R version 3.6.0, pROC package).

Data collection and management

The data collected in this study are summarized in Table 2.

An electronic case report file (e-CRF) will be created for each woman. The women's anonymity will be ensured by mentioning to the maximum extent possible their research code number, followed by the first letters of the last name and first name of the participant on all necessary documents or by deleting their names by appropriate means (white-out) from the copies of source documents intended to document the study.

Table 2: Data collected

Woman characteristic	Age, medical history, parity, contraception, treatment, history of abdominal surgery, smoking
Clinical symptoms	Clinical and/or Ultrasound suspicion, Pelvic pain (type, irradiation, temporality, frequency, associated with nausea or vomiting, previous episode of pain, progression of pain, analgesic intake with dosage), clinical examination (abdominal palpation, vaginal and speculum examination)
Ultrasound signs	Detailed standard pelvic ultrasound examination : Location (right / left), position of the ovaries, sizes, Doppler flow, pain, pelvic effusion, description of a possible pre-existing cystic mass described as IOTA criteria (International Ovarian Tumor Analysis group)
Biomarkers	PCR, White blood cells, Interleukin-6, D-dimere
Contrast ultrasound in the operating room before surgery	Acquisitions performed (MvFlow, Contrast) ; Blood pressive and heart rate during the acquisition , number of contrast injections
Surgical findings	Laparoscopy / laparotomy, confirmed adnexal torsion, number of turns, color of the ovary before detorsion, ovarian cyst, operative step, detorsion, cystectomy, adnexectomy, color of the ovary after detorsion

The data from all women will be centralized and data management will be carried out by Nancy CIC-IT (Inserm CIC 1433). The conditions for data transfer of all or part of the study database are decided by the study sponsor (CHRU de Nancy) and are the subject of a written contract. The data images will be transferred to Nancy CIC-IT and stored after verification, in the ArchiMed database declared to the French authority (CNIL declaration number: 1410005). This study and the data collected fall within the scope of Reference Methodology MR001.

Statistical analysis

The initial characteristics of the women at inclusion will be described in two groups. The quantitative parameters will be described by their means ± standard deviation or median and inter quartile difference according to their normality, maximum and minimum and the qualitative parameters by their numbers and percentages. The normality of the distributions will be checked graphically by histograms and by the Shapiro-Wilk test.

For the main objective, construction and analysis of the ROC curve in order to obtain the signal intensity threshold (determined by the Youden index) to use in order to estimate the sensitivity, specificit, predictive and negative predictive value of contrast enhanced ultrasound. These parameters will be calculated with reference to the surgical intervention.

The overall alpha significance threshold is set at $p<0.05$ in a bilateral situation. Statistical analysis will be performed with R version 4.1.1 or superior. We will not perform an interim analysis.

Patient and Public involvement

Patients and public were not involved.

ETHICS AND DISSEMINATION

The study was approved by the French Ethics Committee, the CPP (Comité de Protection des Personnes) OUEST I on July 3rd, 2020 with reference number 2020T1-16, and the competent authority (Agence Nationale de Sécurité du Médicament et des Produits de Santé) authorized the study on July 16th, 2020. The results of this study will be published in a peer-reviewed journal and will be presented at relevant conferences.

QUALITY CONTROL

Right of access to data and source documents

The Centre Hospitalier Régional Universitaire (CHRU) de NANCY is the sponsor and responsible for obtaining the agreement of all parties involved in the study so as to guarantee direct access source data, source documents, and reports so that the sponsor may control data quality and perform an audit.

Investigators will make available the documents and individual data strictly required for monitoring, quality control and audit of the biomedical study to persons having access to these, in accordance with the statutory and regulatory provisions in place (articles L.1121-3 and R.5121-13 of the French Public Health Code).

Any original document or object that allows the existence or accuracy of a data point or information recorded during the study to be proved is defined as a source document.

In accordance with the statutory provisions in place (articles L.1121-3 and R.5121-13 of the French Public Health Code) the persons having direct access to source data will take every precaution required to ensure the confidentiality of information relating to investigational medicinal products, studies, participants, notably concerning the identity of these, as well as the results obtained. These persons, as the investigators themselves, are subject to professional confidentiality.

During the study, or at its conclusion, data collected regarding participants that is sent to the sponsor by the investigators (or all other specialists involved) will be coded by the inclusion number of the patient in the study. At no point should the names of participants or their addresses appear unencrypted. The presentation of the data processing results can not in any case allow the direct or indirect identification of persons lending themselves to research.

The sponsor will ensure that each study participant has given her written consent for access to her personal data that is strictly required for study quality control.

The data may be transmitted to the companies in the group to which the sponsor belongs and to its contractual partners in a form which should not permit the direct or indirect identification of persons lending themselves to research.

Study monitoring

The monitoring visits (implementation, follow-up and closure) will be performed by the promoting cell of Nancy DRCI (Délégation à la Recherche Clinique et à l'Innovation – CHRU Nancy).

A Clinical Research Associate (CRA) will travel regularly to the centre to perform the quality control of the study.

Depending on monitoring reports and deviations observed, the promoter reserves the right to modify the level of monitoring initially planned.

POTENTIAL RISKS RELATED TO THE STUDY

The only constraint is the completion of two additional ultrasound examinations, with and without injection of contrast: one examination before general anesthesia (on the 2 ovaries) and a second examination after the patient wakes up (only on the ovary with torsion). The timeframe between the SonoVue® injections for the first and second examinations is at least one hour. In case of suspected reaction after the first injection, whether severe or not, the second injection will not be performed.

There are no specific medical risks for women in this study. SonoVue® injection has rare know side effects, usually transient and mild.

ETHICAL PERMISSION

The sponsor and investigators undertake to carry out this research in accordance with the recommendations of the Helsinki Declaration and its revisions, the European Regulation (EU) n° 536/2014 from the European Parliament about clinical trials of medicines for human use, repealing European Directive 2001/20/CE, the n° 2004-806 law of 9 August 2004 about public health policy, the n° 2004-800 law of 6 August 2004 about bioethics, the No 78-17 law of 6 January 1978 relating to data processing, files and freedoms, the n° 2012-300 law of 5 March 2012 about research involving the human person, the 2016-41 law of 26 January 2016 of modernization of our healthcare system and 2016-800 ordinance of 16 June 2016 relating to researches involving the human person and their implementing decrees.
They undertake to comply with all laws and regulations that may apply to research.
The Investigators undertake to respect the protocol in all respects especially with regard to obtaining consent and the notification and follow-up of serious adverse events.

PROTOCOL AMENDMENT

Requests of authorization and/or opinion about substantial amendments will be addressed by sponsor to regulatory institutions.
By signing this protocol, the investigator commits to submit to the Direction of Research and Innovation the substantial amendment project and wait for authorization and/or opinion of regulatory institutions prior to the application of amendment.

FINAL RESEARCH REPORT

The final report of the research will be written collaboratively by the coordinator and the biostatistician mandated for this search. This report will be submitted to each of the investigators for review. Once a consensus has been reached, the final version must be endorsed with the signature of each of the investigators and sent to the sponsor as early as possible after the effective end of the research. A report prepared according to the reference plan of the competent authority must be forwarded to the competent authority and the CPP within a year after the end of the research, understood as being the last follow-up visit of last enrolled subject. This period is abrogated to 90 days in case of premature termination of the research.

DISCUSSION

This study is the first to evaluate ultrasound with contrast injection as a new diagnostic tool in adnexal torsion in adults. Because of the multiple vascularization of the ovary in women, one can hypothesized that contrast will persist but with a flatter intensity curve. The enhancement of the ovarian parenchyma by the contrast product will be decreased by reduction of the arterial flow and will result in the flattening of the initial curve. The final curve may flatten later on due to a decrease in washout, which itself is due to a reduction in venous return linked to the oedema caused by torsion. This phenomenon could be more or less intense depending on the number of turns, but it is also likely that an on/off phenomenon is observed. If this is confirmed by this study, it will be necessary to confirm the interest of this technique on a larger population and in intention-to-treat. The objective of this study is to provide a diagnostic method that is accessible in emergency and inexpensive, allowing the reduction of both the number of false positives and the number of false negatives. Additionally, it would improve the management of women of childbearing age with a suspicion of adnexal torsion. We will also evaluate a functional imaging technique that does not require contrast injection: MvFlow (Microvascular Flow). This technique, if it brings the same results as the contrast one, would bypass the contrast injection and would be even easier to perform in the emergency context.

Author contributions: O.M., G.H., M.B. contributed to the conception and design of the study, R.P., AL.F. and C.B. are the coordinating investigators; A.C. is the study project manager; C.B., R.P. and AL.F. wrote the manuscript; C.B., AL.F., R.P. and K.G. will carry out recruitment, ultrasound acquisition and will collect the data; M.B. is supervising data processing; G.H. is in charge of statistical analysis and all authors reviewed and contributed to the manuscript.
All authors have read, approved the paper and meet the criteria for authorship as established by the International Committee of Medical Journals Editors.

Protocol version: v2.0, 05/11/2020

Funding statement: This work was supported by a grant from the French Ministry of Health (APJ 2019, registration number: N07).

Competing interests statement: None declared

Patient consent: Obtained

Ethics approval: The French Ethics Committee (Comité de Protection des Personnes, CPP) OUEST I approved this study on July 3rd, 2020 (Reference number 2020T1-16).

Data sharing statement: All data generated during this study will be made available via CIC-IT, CHRU Nancy, Nancy- FRANCE in accordance with protocol promotor. Data obtained from this study will be deposited at CIC-IT Nancy where they will be maintained for a minimum of 15 years.

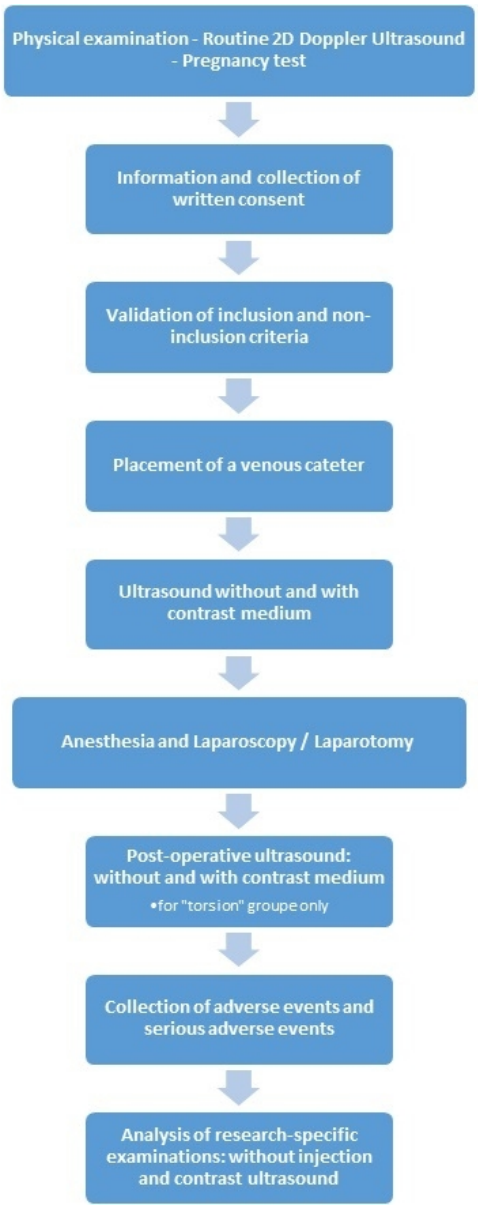
Trial status: This is an ongoing trial. Recruitment began April 13th, 2021. We expect to complete recruitment by April 2024, which is the completion study date. We plan to publish final results in 2024.

REFERENCES

1. Guile SL, Mathai JK. Ovarian Torsion. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.
2. Calis P, Bozdag G, Karakoc Sokmensuer L, Kender N. Does ischemia-reperfusion injury affect ovarian reserve and follicle viability in a rat model with adnexal torsion? Eur J Obstet Gynecol Reprod Biol. févr 2015;185:126-30.
3. Yasa C, Dural O, Bastu E, Zorlu M, Demir O, Ugurlucan FG. Impact of laparoscopic ovarian detorsion on ovarian reserve. J Obstet Gynaecol Res. févr 2017;43(2):298-302.
4. Moro F, Bolomini G, Sibal M, Vijayaraghavan SB, Venkatesh P, Nardelli F, et al. Imaging in gynecological disease (20): clinical and ultrasound characteristics of adnexal torsion. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol. 2020;56(6):934-43.
5. Yatsenko O, Vlachou PA, Glanc P. Predictive Value of Single or Combined Ultrasound Signs in the Diagnosis of Ovarian Torsion. J Ultrasound Med. 2021;40(6):1163-72.
6. Wattar B, Rimmer M, Rogozinska E, Macmillian M, Khan KS, Al Wattar BH. Accuracy of imaging modalities for adnexal torsion: a systematic review and meta-analysis. BJOG Int J Obstet Gynaecol. 2021;128(1):37-44.
7. Duigenan S, Oliva E, Lee SI. Ovarian torsion: diagnostic features on CT and MRI with pathologic correlation. AJR Am J Roentgenol. févr 2012;198(2):W122-131.
8. Paltiel HJ, Estrada CR, Alomari AI, Stamoulis C, Passerotti CC, Meral FC, et al. Multi-planar dynamic contrast-enhanced ultrasound assessment of blood flow in a rabbit model of testicular torsion. Ultrasound Med Biol. févr 2014;40(2):361-70.
9. Trinci M, Danti G, Di Maurizio M, Tursini S, Briganti V, Galluzzo M, et al. Can contrast enhanced ultrasound (CEUS) be useful in the diagnosis of ovarian torsion in pediatric females? A preliminary monocentric experience. J Ultrasound. déc 2021;24(4):505-14.

TABLES AND FIGURES

Table 1: Inclusion and exclusion criteria
Table 2: Data collected
Figure 1: Flow chart of women participation
Figure 2: Ultrasound acquisition in CEUS and in MvFlow



Flow chart of women participation

158x233mm (96 x 96 DPI)

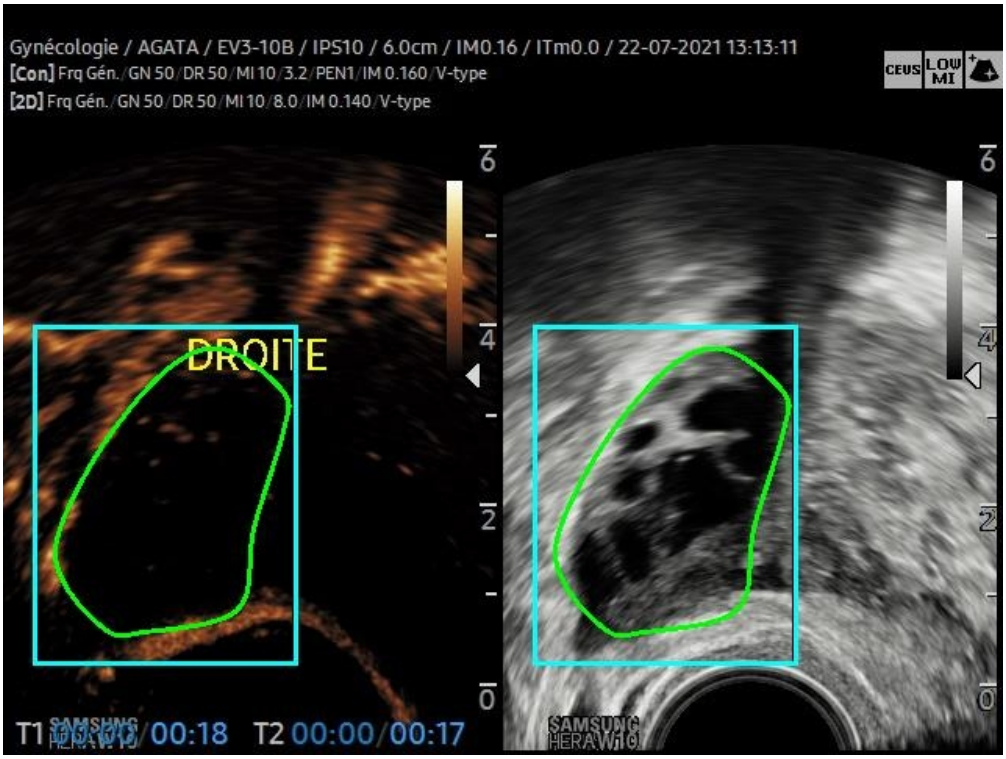


Figure 2 Ultrasound acquisition in CEUS

169x127mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	#3	Date and version identifier	10
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	10

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	7
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	6
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	4

		be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	13
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	5
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	4

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known.	6

		Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	6
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	6
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events	8

		and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	8
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	8
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	7
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	7
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the	7

		public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Annex 1
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

Contribution of contrast enhanced ultrasound in the diagnosis of adnexal torsion (AGATA): protocol for a prospective comparative study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-073301.R1
Article Type:	Protocol
Date Submitted by the Author:	04-Jul-2023
Complete List of Authors:	Pillot, Rémi; CHRU de Nancy Gynécologie obstétrique & néonatologie HOSSU, Gabriela; CHU Nancy, INSERM U1254; CHU Nancy, CIC-IT 1433 CHERIFI, Aboubaker; CHU Nancy, INSERM CIC-IT 1433 Guillez, Kévin; CHRU de Nancy Gynécologie obstétrique & néonatologie, Morel, Olivier; CHRU de Nancy Gynécologie obstétrique & néonatologie Beaumont, Marine; CHU Nancy, Inserm, CIC Fijean, Anne-Laure; CHRU de Nancy Gynécologie obstétrique & néonatologie, Bertholdt, Charline; Obstetric and fetal medicine Unit; IADI
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Radiology and imaging
Keywords:	GYNAECOLOGY, Genitourinary imaging < RADIOLOGY & IMAGING, Ultrasound < RADIOLOGY & IMAGING

Contribution of contrast enhanced ultrasound in the diagnosis of adnexal torsion (AGATA): protocol for a prospective comparative study.

R. Pillot[1], G. Hossu[2,3], A. Cherifi[3], K. Guillez[1], O. Morel[1,2], M. Beaumont[2,3], AL. Fijean[1], C. Bertholdt[1,2]

[1] University of Lorraine, CHRU NANCY, Obstetrics and Gynecology Department, F-54000 NANCY, France
[2] University of Lorraine, INSERM U1254, IADI, F-54000 NANCY, France
[3] CHRU Nancy, Inserm, University of Lorraine, CIC, Innovation Technologique, F-54000 NANCY, France

Corresponding author: AL. Fijean
Obstetric and fetal medicine Unit
Maternité Régionale Universitaire, CHRU de Nancy
10, avenue du Docteur Heydenreich
54000 NANCY, France
Email: a.fijean@chru-nancy.fr

ABSTRACT

Introduction: Adnexal torsion is a surgical emergency and its prognosis depends on the time elapsed prior to treatment. The diagnosis relies on pelvic ultrasound which sensitity remains low and may lead to misdiagnosis.
The primary objective is to evaluate the diagnostic performance of contrast-enhanced ultrasound for the diagnosis of adnexal torsion in women with suspected adnexal torsion. The secondary objectives are: (1) to describe the perfusion parameters of the ovaries by contrast enhanced ultrasound, (2) to compare diagnostic performance of contrast ultrasound with bidimensional Doppler for the detection of adnexal torsion, (3) to describe the perfusion parameters of the ovarian as a function of the degree of adnexal torsion, (4) to compare perfusion parameters before and after ovarian detorsion and (5) to describe perfusion parameters of the ovarian by using MicroVascular Flow™ technique.

Methods and analysis: This is a monocentric, prospective comparative, non randomised, open and interventional study. We hypothesize to include 30 women: 20 positive cases compared to 10 control cases. Women are informed and recruited in the emergency ward, over a period of 36 months.
The primary endpoint is the signal intensity measurement to assess sensitivity, specificity, positive and negative predictive values of contrast-enhanced ultrasound for detection of adnexal torsion in women with suspected adnexal torsion. The presence or absence of adnexal torsion is confirmed during the surgical intervention.

Ethics and dissemination: The study was approved by the French Ethics Committee, the CPP (Comité de Protection des Personnes) OUEST I on July 3rd, 2020 with reference number 2020T1-16. The results of this study will be published in a peer-reviewed journal and will be presented at relevant conferences.

Registration details: ClinicalTrials.gov registry (NCT04522219); EudraCT registry (2020-000993-27).

STRENGTHS AND LIMITATIONS

- This is the first study in adults evaluating the interest of contrast enhanced ultrasound for the diagnosis of adnexal torsion
- This imaging technique has clinical applicability, including in the emergency setting
- This study includes simultaneous evaluation of functional imaging techniques with and without contrast injection
- This study will not be able to assess the potential benefit in the event of false negative, without surgical management
- The sample of patients is small

INTRODUCTION

Adnexal torsion is a surgical emergency due to the total or partial rotation of the adnexa around its vascular axis (the ovary and in rare cases the fallopian tube). Most of cases occur in women of childbearing age and the delay in treatment can lead to ischemia of the adnexa (1). This ischemia may lead to hemorrhagic necrosis of the ovary that could impair its functionality and, consequently, lead to female infertility (2). Treatment is exclusively surgical by laparoscopy or laparotomy and must be performed as soon as possible to preserve ovarian function (3).

Early diagnosis is an essential prerequisite to reduce the potential consequences of ovarian torsion. A short timeframe between the occurrence of torsion and the surgical intervention allows conservative treatment without functional consequences in 90% of cases (2). Usually, this timeframe is approximately 6 hours.

The clinical diagnosis is challenging since the main clinical sign is pelvic pain of sudden onset, a non-specific sign not allowing for a precise diagnosis.

To confirm the diagnosis, pelvic ultrasound with or without Doppler flow analysis is widely used (4). However, its contribution is low, with a sensitivity ranging from 46 to 73% depending on the study and recently confirmed by a meta-analysis (5,6). Other imaging techniques have been considered, such as MRI, with much greater sensitivity than ultrasound. Howeverthe limited accessibility of MRI scanners, particularly in the context of emergency, makes it difficult to use in clinical practice (7,8).

Ultrasound remains the most appropriate modality. SonoVue® ultrasound contrast injection improves blood echogenicity and signal-to-noise ratio (9). This product consists of sulfur hexafluoride microbubbles acting as reflectors of the ultrasound beam with a diameter comparable to the one of a red blood cell (approximately 6 µm): it is a strict intravascular contrast product.

Therefore, contrast-enhanced ultrasound technique seems perfectly suited to assess the vascularisation of the ovary and improve the diagnostic sensitivity of adnexal torsion. Its benefit has already been demonstrated in the diagnosis of testicular torsion in animals, but to date, only one study has evaluated its contribution in adnexal torsion (9,10).

This study by Trinci et al, which assessed the diagnostic performance of contrast-enhanced ultrasound in 20 cases of adnexal torsion, was a retrospective study, performed on a paediatric population. The sensitivity was 94.1% and the specificity was 100%, i.e. an overall accuracy of 95% (10). It confirmed the diagnostic contribution of contrast enhanced ultrasound as a complement to conventional 2D ultrasound in the diagnostic of adnexal torsion.

METHODS AND ANALYSIS

Objectives

The **primary objective** is to evaluate the diagnostic performance of contrast-enhanced ultrasound for the diagnosis of adnexal torsion in women with suspected adnexal torsion.

The **secondary objectives** are:

1. To describe the perfusion parameters of the ovaries by contrast enhanced ultrasound.
2. To compare performance diagnosis of contrast ultrasound with bidimensional Doppler for the detection of adnexal torsion.
3. To describe the perfusion parameters of the ovarian as a function of the degree of adnexal torsion.
4. To compare perfusion parameters before and after ovarian detorsion.
5. To describe perfusion parameters of the ovarian by using MicroVascular Flow™ technique.

Trial design

The AGATA protocol is a monocentric, prospective, comparative, non randomised, open and interventional study.

Study population

Women will be informed and recruited in the emergency ward, over a period of 36 months. All the women suspected of adnexal torsion and with planned surgery who agree to participate in the study will be recruited, despite their medical history and previous examination results. The indication for surgery will be established by the surgeon, at his discretion, on the basis of a number of arguments in favour of adnexal torsion: sudden onset of pain, vomiting, pain not relieved by analgesics, ultrasound signs: enlarged adnexa, presence of an ovarian cyst, ovarian stromal edema, absence of Doppler etc. The inclusion and exclusion criteria are reported in Table 1. The consent collection will be provided by the investigators (obstetricians).

Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Woman over 18 years old • Woman affiliated to a social security • Woman having received complete information on the organization of the research and having given her informed consent in written form. • Planned surgical intervention for suspected adnexal torsion	• Patients under a measure of legal protection, • Contraindication to contrast injection: hypersensitivity to sulfur hexafluoride or any of the other ingredients, history of cardiac disease, respiratory distress syndrome, severe pulmonary hypertension. • Pregnancy

The presence or absence of adnexal torsion was confirmed during the surgical intervention and women were classified retrospectively into two groups: “ovarian torsion” (positives cases) or “no ovarian torsion” (control cases).

Ultrasound acquisition

Ultrasound acquisitions will take place in three stages:

- Identification of ovaries in two-dimensional ultrasound
- Ultrasound acquisitions without injection of contrast product (Microvascular Flow™)
- Ultrasound acquisitions with injection of contrast product (contrast enhanced ultrasound).

These acquisitions will take place at different times of the medical care:

- Before surgery: bilateral acquisition
- After surgery: unilateral acquisition on the affected ovary in case of confirmed torsion.

The flow chart of women participation is in Figure 1.

The ultrasound acquisition will be performed with a HERA W10 (Samsung) and with an endovaginal transducer (3-10 MHz). The ultrasound contrast product used will be Sonovue® (Bracco Imaging, Italy), administered by bolus injection. A volume of 2.4 mL of contrast product will be injected per acquisition with a maximum of 3 injections per women.
The contrast-enhanced acquisition will be performed in contrast mode with standardized parameters. The Microvascular Flow™ acquisition will be performed in MvFlow™ Mode. Dynamic acquisition will be recorded during 2 minutes, and saved as videoclip.

Image analysis

The analysis of contrast-enhanced ultrasound will be performed with a specific off-line software (Vuebox®, 7.0.26 version, Bracco Suisse SA, Geneva, Switzerland). Region of interest will be manually drawn on the overall ovaries allowing to obtain time intensity curve for the quantitative analysis (semiquantitative perfusion indicators).
The analysis of Microvascular Flow™ ultrasound will be performed on the sonographer with a specific integrated software. The region of interest will be drawn, and quantitative parameters will be extract (Figure 2).

Outcomes

The **primary endpoint** is the signal intensity measurement to assess sensitivity, specificity, positive and negative predictive values of contrast-enhanced ultrasound for detection of adnexal torsion in women with suspected adnexal torsion with realization of ROC curves (Receiver Operating Characteristics).

The **secondary endpoints** are:

1. Measurement of perfusion parameters of the suspected ovarian torsion and the contralateral ovary if available: signal intensity and perfusion kinetics.
2. Measurement of signal intensities to assess sensibility and specificity of contrast enhanced ultrasound and bidimensional (2D) Doppler.
3. Comparison of perfusion parameters of the ovary with the degree of torsion. The degree of torsion is defined by the number of twists (number of turns around the axis) detected during the surgical procedure.
4. Measurement of signal intensities before and after ovarian detorsion.
5. Measurement of signal intensities obtained by Micro Vascular Flow™ technique.

Participant timeline

The enrolment of women has started in April 2021. The recruitment should be achieved by April 2024. The flow chart of women participation is presented in Figure 1.

Premature ending of patient participation

Each person can stop participating in the research at any time and for whatever reason.
The investigator may temporarily or permanently interrupt a person's participation in the research for any reason that has an impact on her safety or that would best serve the interests of the person who is suitable for research.
In the event of a premature ending or in the event of consent withdrawal, this shall not affect the activities carried out and the use of data obtained on the basis of informed consent before it has been withdrawn, unless the person indicates in writing that she objects to their use.

Follow-up

A clinical examination will be performed on the inpatient ward at 24 hours by an obstetric gynaecologist to look for any adverse events that have occurred since the inclusion visit.

Sample size consideration

As the research is innovative, no preliminary published figures are available to estimate a number of women to be included. Therefore, we choose to include 30 women. With an assumed distribution of 20 positive cases against 10 control cases, an accuracy of 5% and a power of 95%; this sample will lead to an area under the curve of 85% (R version 3.6.0, pROC package).

Data collection and management

The data to be collected in this study are summarised in Table 2.
An electronic case report file (e-CRF) will be created for each woman. The women's anonymity will be ensured by mentioning to the maximum extent possible their research code number, followed by the first letter of the last name and first name of the participant on all necessary documents or by deleting their names by appropriate means (white-out) from the copies of source documents intended to document the study.

Table 2: Data collected

Woman characteristic	Age, medical history, parity, contraception, treatment, history of abdominal surgery, smoking
Clinical symptoms	Clinical and/or ultrasound suspicion, pelvic pain (type, irradiation, temporality, frequency, associated with nausea or vomiting, previous episode of pain, progression of pain, analgesic intake with dosage), clinical examination (abdominal palpation, vaginal and speculum examination)
Ultrasound signs	Detailed standard pelvic ultrasound examination: location (right / left), position of the ovaries, sizes, Doppler flow, pain, pelvic effusion, description of a possible pre-existing cystic mass described as IOTA criteria (International Ovarian Tumor Analysis group)
Biomarkers	PCR, white blood cells, Interleukin-6, D-dimere
Contrast ultrasound in the operating room before surgery	Acquisitions performed (MvFlow™, Contrast); blood pressive and heart rate during the acquisition , number of contrast injections
Surgical findings	Laparoscopy / laparotomy, confirmed adnexal torsion, number of turns, color of the ovary before detorsion, ovarian cyst, operative step, detorsion, cystectomy, adnexectomy, color of the ovary after detorsion

Data from all women will be centralised and data management will be carried out by Nancy CIC-IT. Conditions for data transfer of all or part of the study database are decided by the study

sponsor (CHRU de Nancy) and will be the subject of a written contract. Imaging data will be transferred to Nancy CIC-IT and stored after verification, in the ArchiMed database declared to the French authority (CNIL declaration number: 1410005). This study and the collected data fall within the scope of Reference Methodology MR001.

Statistical analysis

The initial characteristics of the women at inclusion will be described in two groups. The quantitative parameters will be described by their means ± standard deviation or median and inter quartile difference according to their normality, maximum and minimum and the qualitative parameters by their numbers and percentages. The normality of the distributions will be checked graphically by histograms and by the Shapiro-Wilk test.

For the main objective, construction and analysis of the ROC curve in order to obtain the signal intensity threshold (determined by the Youden index) to use in order to estimate the sensitivity, specificity, predictive and negative predictive values of contrast enhanced ultrasound. These parameters will be calculated with reference to the surgical intervention.

The overall alpha significance threshold is set at $p<0.05$ in a bilateral situation. Statistical analysis will be performed with R version 4.1.1 or superior. We will not perform an interim analysis.

Patient and Public involvement

Patients and public were not involved.

ETHICS AND DISSEMINATION

The study was approved by the French Ethics Committee, the CPP (Comité de Protection des Personnes) OUEST I on July 3rd, 2020 with reference number 2020T1-16, and the competent authority (Agence Nationale de Sécurité du Médicament et des Produits de Santé) authorized the study on July 16th, 2020. The study results will be published in a peer-reviewed journal and will be presented at relevant conferences.

QUALITY CONTROL

Right of access to data and source documents

The Centre Hospitalier Régional Universitaire (CHRU) de Nancy is the sponsor and is responsible for obtaining the agreement of all parties involved in the study so as to guarantee direct access to source data, source documents, and reports so that the sponsor may control data quality and perform an audit.

Investigators will make available the documents and individual data strictly required for monitoring, quality control and audit of the biomedical study to persons having access to these, in accordance with the statutory and regulatory provisions in place (articles L.1121-3 and R.5121-13 of the French Public Health Code).

Any original document or object that allows the existence or accuracy of a data point or information recorded during the study to be proved is defined as a source document.

In accordance with the statutory provisions in place (articles L.1121-3 and R.5121-13 of the French Public Health Code) the persons having direct access to source data will take every precaution required to ensure the confidentiality of information relating to investigational medicinal products, studies, participants, notably concerning the identity of these, as well as the results obtained. These persons, as the investigators themselves, are subject to professional confidentiality.

During the study, or at its conclusion, data collected regarding participants that is sent to the sponsor by the investigators (or all other specialists involved) will be coded by the inclusion number of the patient in the study. At no point should the names of participants or their addresses appear unencrypted. The presentation of the data processing results can not in any case allow the direct or indirect identification of persons lending themselves to research.

The sponsor will ensure that each study participant has given her written consent for access to her personal data that is strictly required for study quality control.

The data may be transmitted to the companies in the group to which the sponsor belongs and to its contractual partners in a form which should not permit the direct or indirect identification of persons lending themselves to research.

Study monitoring

The monitoring visits (implementation, follow-up and closure) will be performed by the promoting cell of Nancy DRCI (Délégation à la Recherche Clinique et à l'Innovation – CHRU Nancy).

A Clinical Research Associate (CRA) will travel regularly to the centres to perform the quality control of the study.

Depending on monitoring reports and deviations observed, the sponsor reserves the right to modify the level of monitoring initially planned.

POTENTIAL RISKS RELATED TO THE STUDY

The only constraint of the study for the participant is the completion of two additional ultrasound examinations, with and without contrast injection: one examination before general anesthesia (on the 2 ovaries) and a second examination after the patient wakes up (only on the ovary with torsion). The timeframe between the SonoVue® injections for the first and second examinations will be at least one hour. In case of suspected reaction after the first injection, whether severe or not, the second injection will not be performed.

There are no specific medical risks for women in this study. SonoVue® injection has rare known side effects, usually transient and mild.

ETHICAL PERMISSION

The sponsor and investigators undertake to carry out this research in accordance with the recommendations of the Helsinki Declaration and its revisions, the European Regulation (EU) n° 536/2014 from the European Parliament about clinical trials of medicines for human use, repealing European Directive 2001/20/CE, the n° 2004-806 law of 9 August 2004 about public health policy, the n° 2004-800 law of 6 August 2004 about bioethics, the No 78-17 law of 6 January 1978 relating to data processing, files and freedoms, the n° 2012-300 law of 5 March 2012 about research involving the human person, the 2016-41 law of 26 January 2016 of modernization of our healthcare system and 2016-800 ordinance of 16 June 2016 relating to researches involving the human person and their implementing decrees.
They undertake to comply with all laws and regulations that may apply to research.
The Investigators undertake to respect the protocol in all respects especially with regard to obtaining consent and the notification and follow-up of serious adverse events.

PROTOCOL AMENDMENT

Requests of authorization and/or opinion about substantial amendments will be addressed by sponsor to regulatory institutions.
By signing this protocol, the investigator commits to submit to the Direction of Research and Innovation the substantial amendment project and wait for authorization and/or opinion of regulatory institutions prior to the application of amendment.

FINAL RESEARCH REPORT

The final report of the research will be written collaboratively by the coordinator and the biostatistician mandated for this search. This report will be submitted to each of the investigators for review. Once a consensus has been reached, the final version must be endorsed with the signature of each of the investigators and sent to the sponsor as early as possible after the effective end of the research. A report prepared according to the reference plan of the competent authority must be forwarded to the competent authority and the CPP within a year after the end of the research, understood as being the last follow-up visit of last enrolled subject. This period is abrogated to 90 days in case of premature termination of the research.

Author contributions: O.M., G.H., M.B. contributed to the conception and design of the study, R.P., AL.F. and C.B. are the coordinating investigators; A.C. is the study project manager; C.B., R.P. and AL.F. wrote the manuscript; C.B., AL.F., R.P. and K.G. will carry out recruitment, ultrasound acquisition and will collect the data; M.B. is supervising data processing; G.H. is in charge of statistical analysis and all authors reviewed and contributed to the manuscript.
All authors have read, approved the paper and meet the criteria for authorship as established by the International Committee of Medical Journals Editors.

Protocol version: v2.0, 05/11/2020

Funding statement: This work was supported by a grant from the French Ministry of Health (APJ 2019, registration number: N07).

Competing interests statement: None declared

Patient consent: Obtained

Ethics approval: The French Ethics Committee (Comité de Protection des Personnes, CPP) OUEST I approved this study on July 3rd, 2020 (Reference number 2020T1-16).

Data sharing statement: All data generated during this study will be made available via CIC-IT, CHRU Nancy, Nancy- FRANCE in accordance with protocol promotor. Data obtained from this study will be deposited at CIC-IT Nancy where they will be maintained for a minimum of 15 years.

Trial status: This is an ongoing trial. Recruitment began April 13th, 2021. We expect to complete recruitment by April 2024, which is the completion study date. We plan to publish final results in 2024.

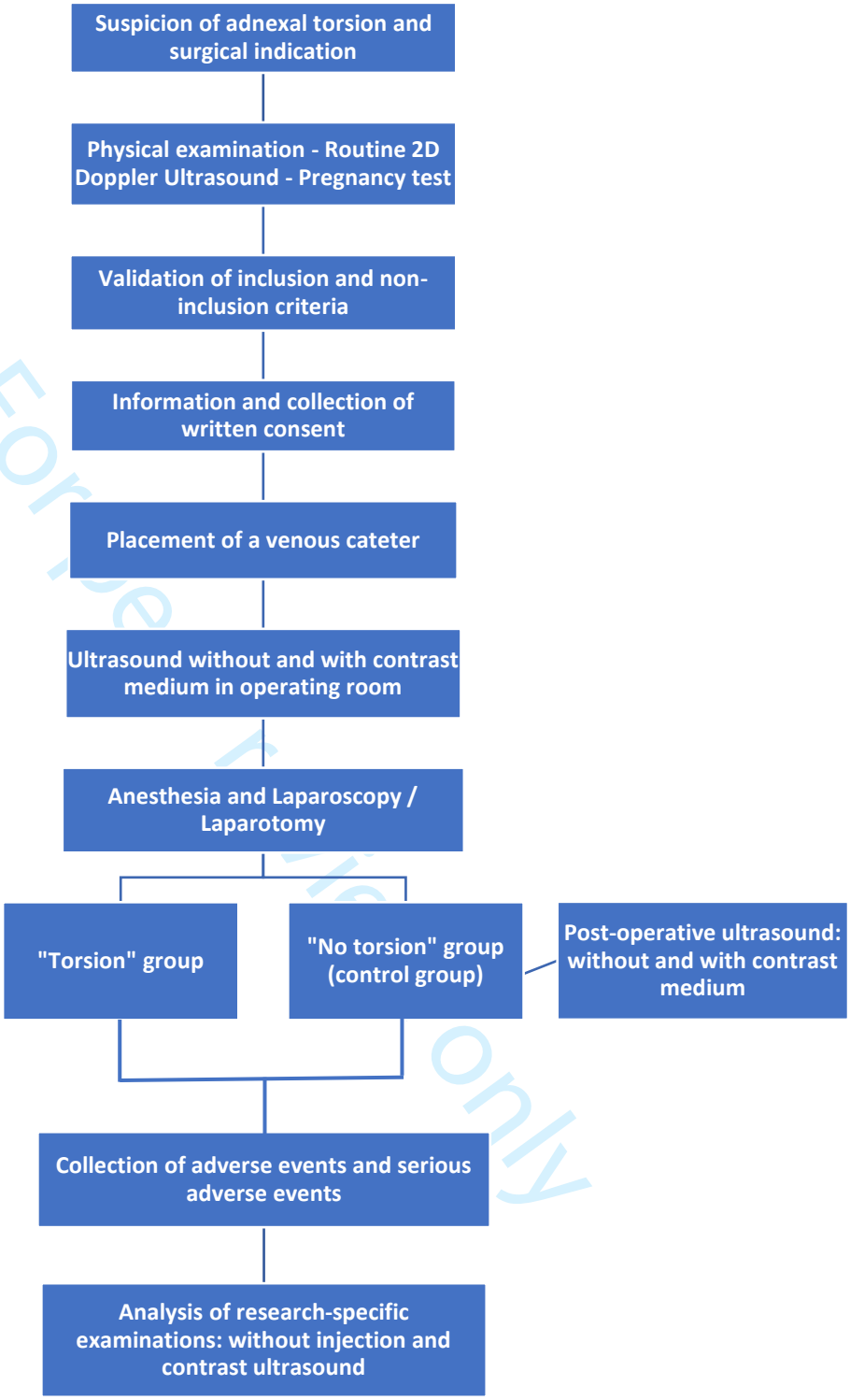
REFERENCES

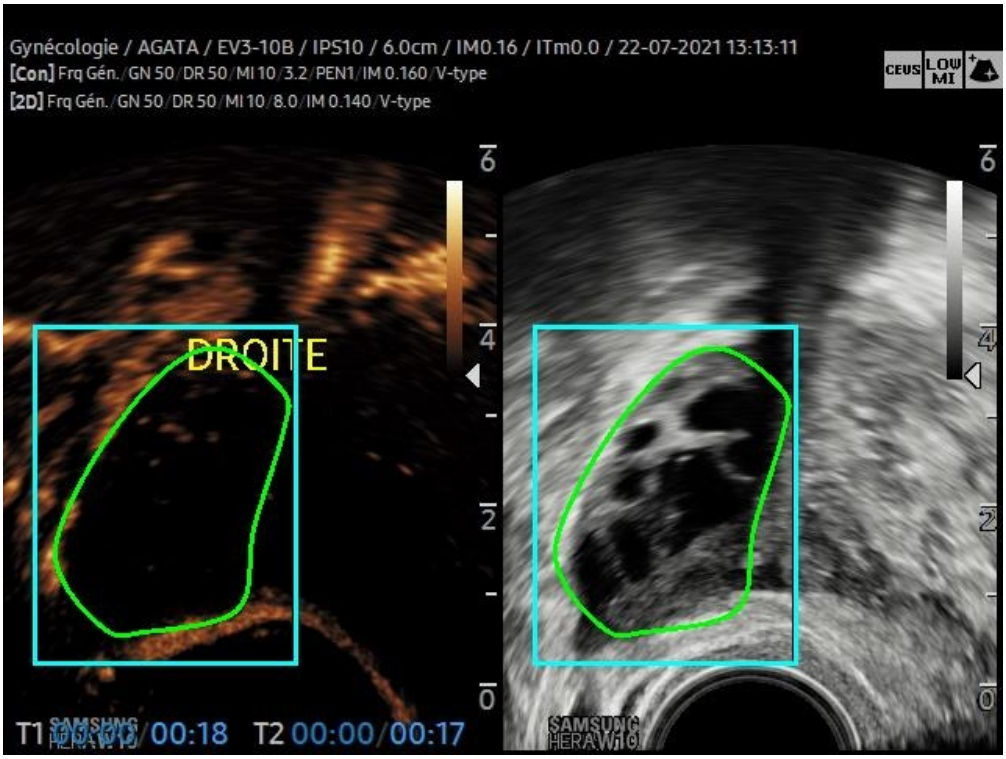
1. Guile SL, Mathai JK. Ovarian Torsion. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Disponible sur: http://www.ncbi.nlm.nih.gov/books/NBK560675/
2. Calis P, Bozdag G, Karakoc Sokmensuer L, Kender N. Does ischemia-reperfusion injury affect ovarian reserve and follicle viability in a rat model with adnexal torsion? Eur J Obstet Gynecol Reprod Biol. févr 2015;185:126-30.
3. Yasa C, Dural O, Bastu E, Zorlu M, Demir O, Ugurlucan FG. Impact of laparoscopic ovarian detorsion on ovarian reserve. J Obstet Gynaecol Res. févr 2017;43(2):298-302.
4. Moro F, Bolomini G, Sibal M, Vijayaraghavan SB, Venkatesh P, Nardelli F, et al. Imaging in gynecological disease (20): clinical and ultrasound characteristics of adnexal torsion. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol. 2020;56(6):934-43.
5. Yatsenko O, Vlachou PA, Glanc P. Predictive Value of Single or Combined Ultrasound Signs in the Diagnosis of Ovarian Torsion. J Ultrasound Med. 2021;40(6):1163-72.
6. Garde I, Paredes C, Ventura L, Pascual MA, Ajossa S, Guerriero S, et al. Diagnostic accuracy of ultrasound signs for detecting adnexal torsion: systematic review and meta-analysis. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol. mars 2023;61(3):310-24.
7. Wattar B, Rimmer M, Rogozinska E, Macmillian M, Khan KS, Al Wattar BH. Accuracy of imaging modalities for adnexal torsion: a systematic review and meta-analysis. BJOG Int J Obstet Gynaecol. 2021;128(1):37-44.
8. Duigenan S, Oliva E, Lee SI. Ovarian torsion: diagnostic features on CT and MRI with pathologic correlation. AJR Am J Roentgenol. févr 2012;198(2):W122-131.
9. Paltiel HJ, Estrada CR, Alomari AI, Stamoulis C, Passerotti CC, Meral FC, et al. Multi-planar dynamic contrast-enhanced ultrasound assessment of blood flow in a rabbit model of testicular torsion. Ultrasound Med Biol. févr 2014;40(2):361-70.
10. Trinci M, Danti G, Di Maurizio M, Tursini S, Briganti V, Galluzzo M, et al. Can contrast enhanced ultrasound (CEUS) be useful in the diagnosis of ovarian torsion in pediatric females? A preliminary monocentric experience. J Ultrasound. déc 2021;24(4):505-14.

TABLES AND FIGURES

Table 1: Inclusion and exclusion criteria
Table 2: Data collected
Figure 1: Flow chart of women participation
Figure 2: Ultrasound acquisition in Contrast-Enhanced Ultrasound and in MvFlow™

Figure 1: Flow chart of women participation





Ultrasound acquisition in Contrast-Enhanced Ultrasound and in MvFlow™

169x127mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	#3	Date and version identifier	10
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	10

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	7
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	6
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	4

		be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	13
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	5
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	4

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known.	6

		Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	6
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	6
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events	8

		and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	8
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	8
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	7
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	7
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the	7

		public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Annex 1
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A