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In vitro fertilisation (IVF) versus intracytoplasmic sperm injection (ICSI) in patients without severe male factor infertility: study protocol for the randomised, controlled, multicentre trial INVICSI

Sine Berntsen ,[1] Bugge Nøhr,[2] Marie Louise Grøndahl,[2] Morten Rønn Petersen,[3] Lars Franch Andersen,[4] Anne Lis Englund,[5] Ulla Breth Knudsen,[6] Lisbeth Prætorius,[1] Anne Zedeler,[1] Henriette Svarre Nielsen,[1,7] Anja Pinborg,[3,7] Nina La Cour Freiesleben[1,7]

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For numbered affiliations see end of article.

Correspondence to
Dr Sine Berntsen;
sineberntsen@gmail.com

ABSTRACT

Introduction Over the last decades, the use of intracytoplasmic sperm injection (ICSI) has increased, even among patients without male factor infertility. The increase has happened even though there is no evidence to support that ICSI results in higher live birth rates compared with conventional in vitro fertilisation (IVF) in cases with nonmale factor infertility. The lack of robust evidence on an advantage of using ICSI over conventional IVF in these patients is problematic since ICSI is more invasive, complex and requires additional resources, time and effort. Therefore, the primary objective of the IVF versus ICSI (INVICSI) study is to determine whether ICSI is superior to standard IVF in patients without severe male factor infertility. The primary outcome measure is first live birth from fresh and frozen-thawed transfers after one stimulated cycle. Secondary outcomes include fertilisation rate, ongoing pregnancy rate, birth weight and congenital anomalies.

Methods and analysis This is a two-armed, multicentre, randomised, controlled trial. In total, 824 couples/women with infertility without severe male factor will be recruited and allocated randomly into two groups (IVF or ICSI) in a 1:1 ratio. Participants will be randomised in variable block sizes and stratified by trial site and age. The main inclusion criteria are (1) no prior IVF/ICSI treatment, (2) male partner sperm with an expected count of minimum 2 million progressive motile spermatozoa following density gradient purification on the day of oocyte pick up and (3) age of the woman between 18 and 42 years.

Ethics and dissemination The study will be performed in accordance with the ethical principles in the Helsinki Declaration. The study is approved by the Scientific Ethical Committee of the Capital Region of Denmark. Study findings will be presented, irrespectively of results at international conferences and submitted for publication in peer-reviewed journals.

Trial registration number NCT04128904. Pre-results.

Strengths and limitations of this study

- This is a randomised controlled trial with concealment of treatment allocation, stratification for age and trial site and use of variable block sizes reducing the risk of selection bias and confounding.
- The large number of subjects included and the multicentre approach of the study increases generalisability of the results.
- The primary outcome is first live birth episode ensuring maximum clinical impact.
- Only first-cycle patients are included to avoid selection bias based on the knowledge of results from previous treatment cycles.
- The study is not blinded neither to study participants nor clinicians, which could potentially introduce bias.

INTRODUCTION

Since the introduction of intracytoplasmic sperm injection (ICSI) in the early 1990s,[1] the use of ICSI has continuously increased and it is now used widely for indications other than male factor infertility. The latest reports from the European Society of Human Reproduction and Embryology (ESHRE) and The International Committee Monitoring-Assisted Reproductive Technologies (ICMART) show that in Europe and globally, ICSI is used in around two-thirds of all fresh-assisted reproductive technology (ART) cycles.[2 3] The ICMART report further accentuates the significant disparities that exists in ART practices across countries. An especially high ICSI:in vitro fertilisation (IVF) ratio is found in the Middle East where the proportion of ICSI cycles in some countries is now 100% of all fresh cycles. It is unlikely that the large disparities

between countries can be explained by differences in the prevalence of male factor infertility alone. In the USA, a recent study, including data from 2000 to 2014, showed a substantial increase (52% increase) in the use of ICSI with no corresponding increase in couples treated for male factor infertility.[4] Likewise, another US study found that the largest increase in the use of ICSI between 1996 and 2012 (from 36% in 1996 to 76% in 2012) was observed among couples without male factor infertility (from 15% to 67%).[5] The observed increase has happened despite the fact that the use of ICSI for nonmale factor infertility remains controversial.[6] While ICSI has resulted in high success rates in couples treated for severe male factor infertility, studies have indicated that ICSI offers no advantage over conventional IVF in nonmale factor infertility couples when it comes to live birth rates.[7–11] Moreover, the American Society for Reproductive Medicine recently published a committee opinion stating that 'in cases without male factor infertility or a history of prior fertilisation failure, the routine use of ICSI for all oocytes is not supported by the available evidence'.[12] In the US study from 2018, the large increase in use of ICSI was correlated with a 7.6% (p=0.001) increase in live birth rates per cycle in women younger than 35 years. When including only data from the most recent years (2008–2014), the correlation between ICSI rates and live birth rates disappeared questioning whether the ICSI method is responsible for the increased live birth rate.[4] The increased use of ICSI without the presence of male factor infertility could be attributed to a general belief that ICSI decreases the risk of fertilisation failure in patients treated for other indications. Indeed, a systematic review and meta-analysis from 2013 reported higher fertilisation rates and a lower risk of fertilisation failure after ICSI compared with conventional IVF in sibling oocytes from patients with unexplained infertility.[13] Yet, many of the included studies did not ascertain their findings with an improvement in clinical outcome (often due to mixed transfers of embryos from IVF and ICSI). Furthermore, other studies find no difference in fertilisation rates or comparable rates of fertilisation failure between the two methods.[14–18] Overall, there is a shortage of randomised controlled trials (RCTs) comparing ICSI and conventional IVF in patients without male factor infertility and the generalisability of findings from existing studies is limited.[19] In an RCT, including 415 patients with nonmale factor infertility, comparable pregnancy rates between ICSI and conventional IVF were observed as well as higher fertilisation rates in the conventional IVF group.[16] Regrettably, live birth rate was not included as an outcome. A large cohort study, including 745 women aged 40 years or older, reported similar live birth rates after ICSI and conventional IVF as well as similar rates of fertilisation and fertilisation failure.[7] Likewise, ICSI does not seem to improve reproductive outcome in women with diminished ovarian reserve (compared with conventional IVF).[20 21] One group of people who might benefit from ICSI are non-male factor infertility patients with a history of total fertilisation failure (or low fertilisation).[22]

In conclusion, there are still significant gaps in the knowledge regarding ICSI versus conventional IVF for couples with normal and nonsevere male factor infertility. Especially when including considerations of cost (either for the individual patient or for the public healthcare system) and complexity of the methods.

The purpose of the IVF versus ICSI (INVICSI) study is to address this knowledge gap and to infer whether ICSI is more effective than standard IVF in patients without severe male factor infertility. The primary outcome measure is first live birth.

METHODS AND ANALYSIS

Hypothesis

ICSI is superior to standard IVF for obtaining live birth of a child in fertility patients without severe male factor infertility.

Study design

The INVICSI study is a multicentre, randomised, controlled trial using a parallel arm design to detect whether ICSI is superior to standard IVF in patients without severe male factor infertility. Patients will be randomised (1:1) to receive insemination of their retrieved eggs with either standard IVF or ICSI. Trial registration data are displayed in table 1. Table 2 provides an overview of revision chronology including current protocol date and version identifier. Protocol modifications are registered continuously on Clinical Trials.gov. The SPIRIT reporting guidelines were used.[23]

Setting

The trial will be conducted in six public fertility clinics in Denmark. All clinics are part of a university hospital setting and all hospitals perform standardised treatments according to the public healthcare system in Denmark. The teams recruiting patients at the trial sites will include fertility doctors, nursing staff and embryologists. Patient enrolment began in November 2019 and will continue until December 2023.

Eligibility criteria

All couples/women referred for their first fertility treatment at six public fertility clinics in Denmark are screened for eligibility with the following inclusion and exclusion criteria:

Inclusion

1. Written informed consent.
2. Age of the woman 18–42 years.
   i. Male partner with normal or non-severely decreased sperm parameters where the semen sample (following density gradient purification) on the day of oocyte pick up (OPU) is expected to contain a minimum of 2 million progressive motile spermatozoa.
   ii. Couples/singles using donor sperm.
3. Body mass index of the woman between 18 kg/m^2 and 35 kg/m^2.
4. First fertility treatment due to:
   i. Tubal factor.

Table 1 Trial registration data

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov ID: NCT04128904, Protocol ID: INVICSI2019
Date of registration in primary registry	10 July 2019
Secondary identifying numbers	H-19022201
Source(s) of monetary or material support	Capital Region of Denmark Gedeon Richter
Primary sponsor	Copenhagen University Hospital Hvidovre
Secondary sponsor(s)	None
Contact for public queries	SB (sineberntsen@gmail.com)
Contact for scientific queries	SB, NCF Department of Obstetrics and Gynaecology The Fertility Clinic, Hvidovre Copenhagen University Hospital Hvidovre
Public title	INVICSI–IVF vs ICSI in patients without severe male factor infertility
Scientific title	IVF vs ICSI in patients without severe male factor infertility (INVICSI): a randomised, controlled, multicentre trial
Countries of recruitment	Denmark
Health condition(s) or problem(s) studied	Methods of insemination (ICSI vs conventional IVF), infertility without severe male factor
Intervention(s)	Active comparator: insemination with ICSI Active comparator: insemination with conventional IVF
Key inclusion and exclusion criteria	Inclusion: age of the woman 18–42 years, BMI of the woman between 18–35 kg/m^2, male partner with normal or non-severely decreased sperm parameters or use of donor sperm Exclusion: previous IVF or ICSI treatments with current partner, use of donor oocytes or frozen oocytes, ovarian cysts>4 cm, known liver or kidney disease, unregulated thyroid disease, endometriosis stage 3–4, hypogonadotropic hypogonadism, other severe comorbidity (eg, diabetes or cardiovascular disease)
Study type	Randomised controlled multicenter trial using a parallel arm design. Randomisation 1:1 to receive insemination with ICSI or conventional IVF
Date of first enrolment	November 29, 2019
Target sample size	824
Recruitment status	Recruiting
Primary outcome(s)	First live birth rate: the number of first live birth episodes from the study oocyte collections including transfer of fresh- and frozen-thawed embryos
Key secondary outcomes	Cycles with total fertilisation failure, fertilisation rate, embryo quality, positive pregnancy test rate, ongoing pregnancy rate, pregnancy loss rate, all live birth episodes, preterm delivery, birth weight and congenital anomalies

ICSI, intracytoplasmic sperm injection; INVICSI, IVF versus ICSI; IVF, in vitro fertilisation.

Table 2 Protocol, revision chronology

Version	Date of approval	Primary reasons for amendment
Original	August 8, 2019	
Amendment 1	January 28, 2020	New trial site added (The Fertility Clinic, Regional Hospital Horsens)
Amendment 2	March 20, 2020	Removed inclusion criteria: (i) regular menstrual cycles (21–35 days). (ii) Diagnostic sperm sample from the male partner with≥4% morphologically normal spermatozoa Added section: handling of poor semen sample on the day of OPU
Amendment 3	September 2, 2020	New trial site added (The Fertility Clinic, Zealand University Hospital)
Amendment 4 (current version)	September 16, 2020	Removed inclusion criteria:treatment with donor sperm or male partner sperm with a minimum concentration of 5 million progressive motile spermatozoa in a (purified) diagnostic semen sample. Added inclusion criteria:male partner with normal or non-severely decreased sperm parameters where the sperm sample (purified) on the day of oocyte pick up is expected to contain a minimum of 2 million progressive spermatozoa.

OPU, oocyte pick up.

ii. Unexplained infertility.
iii. Polycystic ovary syndrome (PCOS).
iv. Light to moderate decreased semen quality in the male partner.

Exclusion

1. Consent not obtained.
2. Significant morbidity in the woman:
   i. Ovarian cysts >4 cm.
   ii. Known liver or kidney disease.
   iii. Unregulated thyroid disease.
   iv. Endometriosis stage 3–4.
   v. Hypogonadotropic hypogonadism.
   vi. Other severe comorbidity (eg, diabetes or cardiovascular disease).
3. Previous IVF or ICSI treatments with current partner.
4. Use of donor oocytes or frozen oocytes.
5. Not speaking or understanding Danish or English language.

Couples using sperm from the male partner as well as couples (or single women) using donor sperm are eligible. Subsequently, randomisation and inclusion will be based on data from the female participant receiving the ovarian stimulation treatment.

The study was originally designed and performed with the additional inclusion criteria of regular menstrual cycles (21–35 days) and a diagnostic sperm sample from the male partner with a minimum of 5 million progressive motile spermatozoa and ≥4% morphologically normal spermatozoa (table 2). However, an amendment was added after the inclusion of 28 participants in May 2020. In this amendment, two of the aforementioned criteria were removed (regular menstrual cycle and minimum percentage of morphological normal sperm). The criterion for sperm morphology was removed because the importance of sperm morphology and whether it should be used to predict fertilisation and reproductive outcome in ART has been questioned.[24–28] The criterion for regular menstrual cycle was removed as current evidence suggests that women with PCOS have similar chances of conceiving with fertility treatment compared with women without PCOS.[29–31]

In September 2019, the criterion for a diagnostic semen sample with a minimum of 5 million progressive motile spermatozoa was also removed (after the inclusion of 88 participants). Due to differences in laboratory techniques and standard tests performed prior to IVF/ICSI on the trial sites, it was not feasible to include a criterion for a diagnostic semen sample. The criterion for number of spermatozoa in the semen sample on the day of OPU remained unchanged.

Screening, inclusion and consent

Potentially eligible patients receive verbal and written information about the study by the investigators during a consultation in the fertility clinic. Inclusion and randomisation of participants to either ICSI or conventional IVF take place after the ovulation trigger has been prescribed and before the oocyte collection. This is to avoid the risk of the allocation group (IVF or ICSI) affecting the clinicians' choice when deciding the dose of the follicle-stimulating hormone as well as the timing (or cancellation) of oocyte collection. Also, this ensures that the decision for inclusion is not based on the number of oocytes collected. Couples/women who wish to participate in the trial are asked to sign an informed consent form prior to enrolment. They will usually have a minimum of 2 days between receiving the information and deciding whether they wish to participate in the study or not. When a patient has given consent and inclusion criteria are met, randomisation is conducted in the online platform REDCap, which is also used for data collection during the study.[32] The REDCap database has a complete audit trail and is based on anonymous subject ID numbers. It is not revealed whether the patient is assigned to standard IVF or ICSI until after the patient has been recruited and baseline data have been entered in REDCap ensuring treatment allocation concealment. Participants can withdraw from

the trial at any time without giving an explanation, and their fertility treatment will not be affected.

Randomisation

An independent statistician prepared the computer-generated randomisation scheme in a I:I ratio between the two arms (IVF and ICSI). Permuted blocks of variable size between 4 and 12 were used for randomisation. The randomisation scheme was stratified by trial site and female age (three age groups: 18–25 years of age, 26–37 years of age and 38–41 years of age) to ensure that the number of participants receiving IVF and ICSI is closely balanced within each stratum. The randomisation procedure is performed online in REDCap. The allocation table was uploaded in REDCap by the independent statistician and concealed from the clinical staff performing the randomisation. The unique Danish social security number of each participant is entered initially ensuring that no participants are randomised two times.

Poor semen sample on the day of OPU

If the purified semen sample contains less than 2 million progressive spermatozoa on the day of OPU, the woman/couple will be treated with ICSI regardless of allocation.

Blinding

The study is designed with no blinding of participants, clinicians or assessors. It was decided not to blind clinicians and participants as our experience shows that patients in the Danish fertility clinics are eager to know the insemination method used in their treatment. Hence, it was deemed unrealistic to recruit participants if allocation was only revealed after the endpoints were reached.

Intervention

The participants will receive conventional IVF or ICSI treatment as determined by randomisation. Both treatments are part of standard treatment regimens at the trial sites.

The fertility treatment

The women have been treated in either a short gonadotropin-releasing hormone (GnRH)-antagonist protocol or a long GnRH-agonist protocol for ovarian stimulation. The controlled ovarian stimulation, transvaginal ultrasound examinations and the ovulation triggering are done according to the usual daily practice at the trial sites which normally entails ovulation trigger being prescribed when a minimum of two to three follicles measure 17 mm or more. However, women with only one mature follicle may also be prescribed the ovulation trigger. OPU is performed 36±2 hours after the ovulation trigger is administered. On the day of OPU, the concentrations of all spermatozoa and progressive motile spermatozoa is assessed in the ejaculate. Following density gradient purification, wash steps and resuspension in 1 mL media, the number of all spermatozoa as well as the number of progressive motile spermatozoa are assessed again. In cases with a high concentration of spermatozoa in the ejaculate, it is allowed to purify only part of the sample. In this case, a theoretical (after purification) total yield is calculated.

Oocyte insemination will be IVF or ICSI according to randomisation, using established procedures at the trial sites. However, short time insemination in the IVF arm is not allowed. In case of total fertilisation failure, rescue ICSI is not performed. Embryo culture and luteal phase support will follow the usual procedures at each trial site. Blastocyst transfer is performed on day 5. Patients with a poor ovarian reserve and few oocytes retrieved (≤4) are allowed transfer day 2 or 3 according to clinical practice. Single embryo transfers are planned. Surplus blastocysts of good quality are vitrified on day 5 or 6. Transfer and cryopreservation are done according to usual practice at each trial site. In cases with total freeze of all blastocysts due to the risk of ovarian hyperstimulation syndrome, women are not excluded from the trial. In cases where all blastocysts or spare blastocysts are vitrified, these are transferred in subsequent frozen-thawed embryo transfer (FET) cycles according to the daily practice at each trial site (ie, natural cycles, substituted or stimulated FET cycles).

Urine pregnancy test or a serum pregnancy test is done at 11–16 days after embryo transfer. If pregnancy is achieved, a transvaginal ultrasound scan is performed at pregnancy week 7–9 to confirm an ongoing and intrauterine pregnancy.

Women will be asked to inform the clinic of the result of the pregnancy as is the usual procedure in the clinic.

Study outcomes

Primary endpoint

The primary endpoint for the INVICSI trial is the first live birth episode following the study cycle in each of the two groups (IVF and ICSI). This is defined as the first live birth from the oocyte collection and includes transfer of fresh embryos and frozen-thawed embryos. The minimum follow-up time will be 1 year after inclusion. Live birth is defined as the delivery of one or more living infants ≥22 weeks of gestation. When the primary endpoint is achieved, further live births from the oocyte collection will not be included in the primary outcome analysis. Subsequent live births from any FET cycles with embryos from the first fresh cycle are included as a secondary outcome (all live birth episodes). The secondary outcomes are summarised in table 3.

Data collection methods

Before treatment is initiated all fertility patients in the clinics fill out a standard form including data on fertility and medical history, ethnicity, medications, smoking, alcohol, height, weight and so on. These data are routinely entered into electronic medical files of the fertility clinics by fertility doctors prior to the patients first consultation in the clinic. This is part of standard practice for all fertility patients. For the INVICSI study, baseline data will be gathered by the investigators from the

Table 3 Secondary outcomes

Outcome	Assessment
Fertilisation	Fertilisation rate per aspirated oocyte retrieved (16–20 hours after IVF/ICSI) defined as the appearance of 2 pronuclei
	Cycles with total fertilisation failure
Embryo data	Embryo quality (ie, good quality blastocysts according to Gardner classification)
	Embryo time-lapse kinetics including cleavage patterns
	Embryo utilisation rate (number of transferred + cryopreserved embryos per number of 2 PN zygotes)
Freeze	Number of frozen blastocysts (time frame: up to 6 days after oocyte pick up (OPU))
Pregnancy	Positive pregnancy test (positive urine or serum hCG 11-21 days after embryo transfer)
	Multiple pregnancy (period: up to 12 weeks after embryo transfer). Number of intrauterine gestations
	Ongoing pregnancy per transfer (fetal heartbeat on ultrasound in gestational week 7–8)
Miscarriage	Pregnancy loss rate (period: up to 12 weeks after embryo transfer)
	Biochemical pregnancies (positive urine or serum hCG 11-21 days after embryo transfer without any clinical signs of intra- or extrauterine pregnancy)
	Ectopic pregnancy/PUL
Birth/offspring	All live birth episodes (all live births from the study oocyte collection (including second and further live births)
	Preterm delivery (delivery at gestational week 22–36+6)
	Birth weight/weight for gestational age
	Congenital anomaly diagnosed at birth

ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilisation; OPU, oocyte pick up; PN, pronuclei; PUL, pregnancy of unknown location.

electronic files after written informed consent has been given (age, weight, height, ethnicity, antral follicle count, antimüllerian hormone concentration, years of infertility, primary or secondary infertility, infertility diagnosis, stimulation protocol, sperm characteristics). Data will then be entered into REDCap after which the randomisation and allocation to either standard IVF or ICSI will occur. Data on treatment outcome including fertilisation, embryo development, pregnancy and pregnancy loss (secondary outcomes, table 3) will be collected and entered in REDCap. The couple/woman is asked to consent to data being obtained from the child's file in case the fertility treatment results in the birth of a living child.

To ensure data collection, an investigator will follow-up on all participants who get pregnancy. Follow-up will take place 1 year after the ultrasound scan (weeks 7–9). If the participant has informed the fertility clinic on birth and child, an investigator will contact the participant via a phone call or retrieve all information from the electronic patient record.

Statistical considerations

Proposed sample size

The rate of first live births after transfer of up to all of the transferable embryos from the first OPU is set to 45% in the conventional IVF group and 55% in the ICSI group. This is a superiority trial with a power of 80% and a two-sided p value of 5%. The sample size is estimated to be 392 patients in each group. Postrandomisation exclusion is expected to be 5%, resulting in a total of 824 patients.

Data analysis

ITT (Intention-To-Treat) analysis and per-protocol analysis will be performed. Baseline characteristics and outcomes will be compared using t-test, Mann-Whitney U test or χ tests for continuous and categorical variables or logistic regression analysis, controlling for possible confounding effects where appropriate. P values of <0.05 will be considered statistically significant. Statistical analyses will be performed by an investigator together with statistical experts. The primary RCT analysis will be performed by an independent statistician blinded to group allocation.

Ethics and dissemination

Data security and ethical aspects

Data to describe the study population and the outcomes will be collected in a single database including all participants with an identification code, which makes every participant anonymous in the database.

The study is approved by the Scientific Ethical Committee of the Capital Region of Denmark (H-19022201) and the Danish Knowledge Centre on Data Protection Compliance. The study will be performed according to the Danish Law and Ethical principles in the Helsinki Declaration. Each participant will receive oral and written information about the study and will have opportunity for time and reflection. They can also discuss their participation with a third person. The collected oocytes of the participants will be fertilised with IVF or ICSI according

to randomisation. Some couples/women may experience no fertilisation after either IVF or ICSI in the study. This risk is not considered higher compared with women who do not participate in the study. The study is registered with the National Institute of Health's ClinicalTrials.gov.

Dissemination

The findings of the study will be presented at national and international fertility conferences such as the ESHRE annual meeting. In addition, the findings will be published in peer-reviewed scientific journals. Public dissemination will be in the lay press.

DISCUSSION

Worldwide, the rate of treatment cycles where oocytes are fertilised with ICSI is increasing, also in patients without severe male factor infertility. Currently, there is no evidence to support that ICSI results in a higher live birth rate compared with standard IVF in these patients. If the INVICSI study finds that ICSI is superior to standard IVF in cases without severe male factor infertility, the increase in use of ICSI is justified and may then be recommended. However, if the INVICSI study fails to show superiority of ICSI, standard IVF should be recommended as the preferred first choice method of fertilisation in patients without severe male factor infertility. This could potentially lead to significant cost savings and a higher use of standard IVF that is less invasive, closer to natural fertilisation and less expensive.

Author affiliations
[1]Department of Obstetrics and Gynaecology, The Fertility Clinic, Copenhagen University Hospital Hvidovre, Hvidovre Hospital, Hvidovre, Denmark
[2]Department of Obstetrics and Gynaecology, The Fertility Clinic, Copenhagen University Hospital Herlev, Herlev Hospital, Herlev, Denmark
[3]The Fertility Clinic, Copenhagen University Hospital Rigshospitalet, Rigshospitalet, Copenhagen, Denmark
[4]Department of Obstetrics and Gynaecology, Copenhagen University Hospital North Zealand, North Zealand Hospital, Hilleroed, Denmark
[5]Department of Obstetrics and Gynaecology, The Fertility Clinic, Zealand University Hospital Koege, Zealand University Hospital Koge, Koege, Denmark
[6]Department of Obstetrics and Gynaecology, The Fertility Clinic, The Regional Hospital Horsens, Regional Hospital Horsens, Horsens, Denmark
[7]Institute of Clinical Medicine, University of Copenhagen Faculty of Health and Medical Sciences, Copenhagen, Denmark

Contributors SB and NLCF were responsible for the conception, design and execution of the study protocol. SB, NLCF and AP contributed to the initial revision and editing of the manuscript. AZ was consulted concerning the laboratory details of the study design. SB, NLCF, AP, AZ, ALM, UBK, MRP, LFA, BN, HSN, LP and MLG contributed to the critical revision of the manuscript as well as the approval of the final version for submission in BMJ Open.

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ORCID iD
Sine Berntsen http://orcid.org/0000-0001-9769-5002

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