BMJ Open

Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: Protocol for a randomized, double-blind, placebo-controlled trial (The BREAST-AB trial)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-058697
Article Type:	Protocol
Date Submitted by the Author:	27-Oct-2021
Complete List of Authors:	Hemmingsen, Mathilde; Rigshospitalet, Department of Plastic Surgery and Burns Treatment Larsen, Andreas; Rigshospitalet, Department of Plastic Surgery and Burns Treatment Weltz, Tim; Rigshospitalet, Department of Plastic Surgery and Burns Treatment Ørholt, Mathias; Rigshospitalet, Department of Plastic Surgery and Burns Treatment Wiberg, Sebastian; Rigshospitalet, Department of Cardiology Bennedsen, Anne; Rigshospitalet, Department of Plastic Surgery and Burns Treatment Bille, Camilla; Odense University Hospital, Department of Plastic Reconstructive Surgery Carstensen, Lena; Southwest Jutland Hospital, Department of Plastic Surgery Jensen, Lisa; Rigshospitalet, Department of Plastic Surgery and Burns Treatment Hölmich, Lisbet; Herlev Hospital, Department of Plastic and Reconstructive Surgery; University of Copenhagen, Department of Clinical Medicine Bredgaard, Rikke; Rigshospitalet, Department of Plastic Surgery and Burns Treatment Koudahl, Vibeke; Lillebaelt Hospital, Department of Plastic Surgery Schmidt, Volker ; Zealand University Hospital Roskilde, Department of Plastic and Breast Surgery; University of Copenhagen, Department of Clinical Medicine Vester-Glowinski, Peter; Rigshospitalet, Department of Plastic Surgery and Burns Treatment Sørensen, Søren; University of Copenhagen, Department of Biology, Section of Microbiology Bjarnsholt, Thomas; University of Copenhagen, Department of Immunology and Microbiology; Rigshospitalet, Department of Clinical Microbiology Damsgaard, Tine; Rigshospitalet Klinik for Plastikkirurgi Brystkirurgi og Brandsarsbehandling, Department of Plastic Surgery and Burns Treatment; University of Copenhagen Faculty of Health and Medical Sciences, Institute of Clinical Medicine, University of Copenhagen

	Herly, Mikkel; Rigshospitalet, Department of Plastic Surgery and Burns Treatment; University of Copenhagen, Department of Clinical Microbiology
Keywords:	Plastic & reconstructive surgery < SURGERY, Breast surgery < SURGERY, Clinical trials < THERAPEUTICS

Licence statement

Title: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: Protocol for a randomized, double-blind, placebo-controlled trial (The BREAST-AB trial)

Authors

Mathilde N Hemmingsen, MD[1]; Andreas Larsen, MD[1]; Tim K Weltz, BMSc[1]; Mathias Ørholt, MD[1]; Sebastian Wiberg, MD[2]; Anne K Bennedsen, BMSc[1]; Camilla Bille, MD[3]; Lena F Carstensen, MD[4]; Lisa Toft Jensen, MD[1]; Lisbet Rosenkrantz Hölmich[5,6], MD; Rikke Bredgaard, MD[1]; Vibeke Koudahl, MD[7]; Volker J Schmidt, MD[8,6]; Peter Vester-Glowinski, MD[1]; Søren J Sørensen, MSc[9]; Thomas Bjarnsholt, MSc[10,11]; Tine Engberg Damsgaard, MD[1,6]; Mikkel Herly, MD[1,10]

Affiliations

[1]Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

[2]Department of Cardiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

[3]Department of Plastic Reconstructive Surgery, Odense University Hospital, Odense, Denmark

[4]Department of Plastic Surgery, Southwest Jutland Hospital, Esbjerg, Denmark

[5]Department of Plastic and Reconstructive Surgery, Copenhagen University Hospital, Herlev and Gentofte, Copenhagen, Denmark

[6]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

[7]Department of Plastic Surgery, Lillebaelt Hospital, Vejle, Denmark

[8]Department of Plastic and Breast Surgery, Zealand University Hospital, Roskilde, Denmark

[9]Section of Microbiology, Department of Biology, University of Copenhagen, Copenhagen, Denmark

[10]Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

[11]Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

Corresponding author

Mathilde Nejrup Hemmingsen, MD

Department of Plastic Surgery and Burns Treatment

Copenhagen University Hospital, Rigshospitalet

Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Email: mathilde.nejrup.hemmingsen@regionh.dk

Phone: +45 285 682 05

Abstract (251/300)

Introduction

Periprosthetic infection is one of the most severe complications to implant-based breast reconstruction affecting 5-10% of the women. Currently, many surgeons soak the breast implant in antibiotics to reduce the risk of postoperative complications, but there are no randomized, placebo-controlled trials on whether the treatment prevents implant infection and implant loss.

Methods and analysis

The BREAST-AB trial is an investigator-initiated, multicentre, randomized, placebo-controlled, double-blind trial of local treatment with gentamicin, vancomycin and cefazolin in women undergoing implant-based breast reconstruction. The trial drug consists of 80 mg gentamicin, 1 g vancomycin and 1 g cefazolin dissolved in 500 ml of isotonic saline. The placebo solution consists of 500 ml isotonic saline. During the surgery, the trial drug is used to wash the dissected tissue pocket and the breast implant prior to insertion. The primary outcome is all-cause explantation within 180-days after the breast reconstruction surgery. Key secondary outcomes include surgical site infection and revisional surgery. Long-term outcomes include capsular contracture and quality of life. The trial started on 26 January 2021 and is currently recruiting.

Ethics and dissemination

The trial was approved by the Regional Ethics Committee of the Capital Region (H-20056592) on 1 January 2021 and the Danish Medicines Agency (2020070016) on 2 August 2020. The main paper will include the primary and secondary outcomes and will be submitted to an international peer-reviewed journal.

Registration

The trial was registered at ClinicalTrials.gov (NCT 04731025) on 29 January 2021 and at the EU Clinical Trials Register (EudraCT 2020-002459-40) on 17 December 2020.

Strengths and limitations of this study

- This is the first randomized, placebo-controlled clinical trial to investigate the effect of locally applied antibiotics on all-cause loss of the implant after implant-based breast reconstruction
- The primary outcome is patient-oriented and the results from the trial will therefore have a direct impact on patients undergoing implant-based breast reconstruction
- The paired design in patients undergoing bilateral surgery increases the statistical power because the patient acts as her own control and the effect of the trial drug is isolated from the inter-individual variation
- The results from this trial may contribute to international consensus guidelines regarding the use of locally applied antibiotics for implant-based breast reconstruction
- The incidence of the primary outcome is relatively low, so despite the large sample size, a small effect of the treatment may not be detected with statistical significance

Text (2.331/4.000 words)

Introduction

Breast reconstruction has been shown to improve a women's quality of life after being diagnosed with breast cancer.[1] An increasing number of women choose implant-based breast reconstruction[2] which includes a risk of implant infection. Implant infection is seen in 5-10% of the women,[3–7] and the treatment typically requires removal of the implant after which the patient must wait several months before a new breast reconstruction can be attempted.

Previous studies suggest that bacterial contamination of the breast implant can occur without any clinical symptoms.[8,9] Instead, the bacteria form a chronic, subclinical infection which is suspected to cause a prolonged immune reaction to the implant called capsular contracture which affects up to 10-20% of the patients.[10,11] Capsular contracture causes hardening and deformity of the breast, and the treatment often includes surgical removal of the contracted capsule and exchange of the implant.

Surgeons have attempted numerous strategies to prevent bacterial contamination of the implant.[12–17] The most widely followed approach is to apply antibiotics directly on the breast implant and in the dissected tissue pocket during the surgery,[18] but only a few studies have investigated the clinical effect of the treatment. A recent meta-analysis investigating the clinical effect of locally applied antibiotics found a decreased rate of implant infection and capsular contracture in women treated with antibiotics applied on the breast implant.[19] However, the included studies were mostly retrospective with heterogeneity in the applied antibiotics, control groups and follow-up period. The meta-analysis did not identify any randomized controlled trials.[19]

Due to the limited evidence, there are no official clinical recommendations regarding the use of locally applied antibiotics on breast implants. The National Institute for Health and Care Excellence (NICE) in England have requested further studies investigating the clinical effect of locally applied antibiotics on implants.[20] Randomized clinical trials are essential for developing evidence-based treatment guidelines.

The BREAST-AB trial is designed to assess the effect of locally applied antibiotics on all-cause loss of the implant after implant-based breast reconstruction. We hypothesize that local application of

gentamicin, vancomycin and cefazolin will decrease the risk of postoperative clinical infections and thereby reduce the risk of losing the implant to the benefit of women undergoing implant-based breast reconstruction.

Methods and analysis

The protocol was made in accordance with the SPIRIT statement[21] and the ICH-GCP guidelines[22]. The protocol is provided in full length in the supplemental material.

Trial design

The BREAST-AB trial is an investigator-initiated, multicentre, randomized, double-blind, placebo-controlled trial investigating local application of gentamicin, vancomycin and cefazolin during implant-based breast reconstruction. The antibiotic solution or placebo is applied directly onto the breast implant and in the dissected tissue pocket during the surgery.

Setting

The trial will be conducted at six hospitals in Denmark, and additional trial sites may be included during the trial period. A list of the trial sites is provided in the full protocol in the supplemental material.

Eligibility criteria

Patients that meet the following criteria are considered eligible for inclusion:

- Age ≥ 18
- Biologically female
- Written informed consent
- Scheduled for breast reconstruction with implants or expanders including
  - immediate or delayed reconstruction
  - unilateral or bilateral reconstruction
  - with or without simultaneous flap reconstruction

Exclusion criteria are:

- Pregnancy

- Breast feeding
- Known allergy towards gentamicin, vancomycin, cefazolin or neomycin
- Known anaphylactic reaction towards beta-lactam antibiotics or aminoglycosides
- Myasthenia gravis
- Known impaired renal function < 60 ml/min
- Participation in investigational drug trials concerning disinfection agents in the breast cavity

Trial intervention

The trial drug will contain 80 mg gentamicin, 1000 mg vancomycin and 1000 mg cefazolin dissolved in an infusion bag containing 500 ml of sterile isotonic saline. The placebo solution will consist of 500 ml sterile isotonic saline contained in a similar infusion bag. Both solutions are achromatic, and the infusion bags are indistinguishable from one another. See figure 1 for an illustration of the trial intervention.

During the surgery, the responsible nurse draws 150 ml from the assigned infusion bag and the plastic surgeon use it to wash the dissected tissue pocket. Another 50 ml is drawn from the same infusion bag and used to soak the implant prior to insertion in the tissue pocket. The rest of the content in the infusion bag is discarded.

Randomization

The trial drug and placebo are assigned in a 1:1 ratio. Patients undergoing unilateral breast reconstruction will be randomized to either the trial drug or placebo, whereas patients who undergo bilateral breast reconstruction will be randomized to the trial drug on one breast and placebo on the contralateral breast. The paired design in the patients undergoing bilateral surgery isolates the effect of the trial treatment from the inter-individual variation as the patients serve as their own control. Patients who undergo two-stage breast reconstruction with an expander implant which is replaced with a permanent implant after three to six months will be allocated to the same trial treatment during both surgeries. See figure 2 for an overview of the trial design.

The randomization is stratified according to study site, whether the patients undergo unilateral or bilateral surgery and selected risk factors based on the literature[23] including radiation therapy and

immediate versus delayed reconstruction. This approach will ensure an even distribution of the selected risk factors for the outcomes in the placebo group and the intervention group. The randomized design will ensure that other potential risk factors, which are not included in the stratification, are evenly distributed in the intervention- and control group. The treatment is assigned in a fixed block size of two to ensure that the trial drug and placebo is evenly distributed within each stratum.

Blinding

The trial is double-blind so that the patients, site investigators, health care personnel and the data assessors are blinded to the allocated treatment. The only unblinded investigators are the nurses responsible for preparing the trial drugs and the members of the trial coordination unit who provide the treatment allocation. The unblinded investigators do not take part in any treatment-related procedures, clinical evaluation of the outcomes or data assessment. In case of emergency unblinding, the trial coordination unit will provide the allocation assignment under discretion of the treating physician.

Primary outcome

- All-cause explantation of the breast implant within 180-days after the breast reconstruction

All-cause explantation is defined as surgical removal of the implant without replacing it with a new implant. The rationale for the primary outcome is to quantify whether the locally applied antibiotics prevents severe infection that leads to loss of the implant. Sometimes, the indication for explantation of the implant may be ambiguous because multiple complication can occur simultaneously. Therefore, all-cause explantation was chosen as a more objective alternative to infection that leads to explantation of the implant.

Secondary outcomes

- Time to explantation

Time to explantation is defined as the amount of days from the reconstructive surgery to the surgical removal of the implant. This outcome was chosen because application of local antibiotics may delay the development of a postoperative clinical infection.

- Revision surgery with incision of the fibrous capsule after the breast reconstruction surgery

This is defined as all revisional surgery that includes exposure of the breast implant. This outcome was included because the breast reconstruction in some cases can be upheld with revisional surgery despite complications that may be associated with low-virulent bacteria.

- Exchange of the permanent implant with an expander implant after the breast reconstructive surgery

In some cases, the reconstructed breast can be upheld by exchanging the permanent implant with an expander implant which allows for stepwise expansion and preserved vitality of the vulnerable skin flaps until a permanent implant can be inserted.

- Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction

Surgical site infection is defined according to the CDC classification.[24] The clinical signs of infection is combined with the prescription of antibiotics as a confirmation of the surgeons suspicion of infection. Additional outcome measures are listed in the supplemental material and on ClinicalTrials.gov.

Long-term outcomes

The trial includes a long-term assessment of capsular contracture after 5, 10 and 15 years. The use of locally applied antibiotics could potentially decrease the rate of capsular contracture by minimizing the low-virulent bacterial contamination of the implant. Therefore, capsular contracture is an important long-term outcome.

The trial also evaluates long-term quality of life using the BREAST-Q questionnaire.[25] The application of local antibiotics may decrease the risk of postoperative complications and thereby decrease the risk of undergoing revision surgery. This in turn may lead to improved patient

satisfaction and quality of life. BREAST-Q is a validated tool used to quantify patient satisfaction and health-related quality of life after breast reconstruction surgery.

Sample size

The trial is powered to find a 5% risk reduction in the primary outcome. Based on the literature, the assumed rate of implant loss in the control group is 10%.[6,7] The independent sample unit is "breast", because previous data does not suggest that implant loss is correlated between the two breasts of a patient.[3] Therefore, the power of the trial is based on the number of breasts, so that the final number of included patients depends on the proportion of patients who undergo bilateral breast reconstruction. With an alpha of 0.05, the trial will have a power of 0.90 to detect an absolute risk reduction of 5% with 1158 breasts included. To account for drop-out of up to 10%, recruitment will continue until patients with a combined number of 1274 breasts are included in the trial. This is estimated to correspond to 1003 patients, if approximately 27% of the patients undergo bilateral breast reconstruction.

Statistical analysis plan

The statistical analyses and reporting will adhere to the CONSORT guidelines.[26] All statistical analyses will be conducted on a modified intention-to-treat population defined as all patients that have been allocated to the study drug and have a valid informed consent.

The primary outcome and key secondary outcomes are categorical variables and will be presented as frequencies in each group. The overall effect of the intervention on the primary and secondary outcomes will be modelled as both univariate and multivariate mixed effects logistic regression models considering the correlation between breasts in patients undergoing bilateral surgery. The results will be presented as crude and adjusted odds ratios (OR) with 95% confidence intervals. The model will be adjusted for potential confounders, including age, smoking, body mass index, trial site and indication for surgery (prophylactic mastectomy versus mastectomy after cancer diagnosis). The full statistical analysis plan is provided in the protocol in the supplemental material.

Data collection and follow-up

All patients are admitted at the hospital for approximately 3 days after the surgery. All patients are scheduled for postoperative follow-up visits after approximately 3 months and 1 year. Data on drug administration is obtained real-time and entered in an electronic case report form. Additional data is obtained from the patient's medical records by trained researchers and entered in the electronic case report form. A list of included variables is provided in the protocol in the supplementary material.

Clinical treatment

Participation in the trial will not interfere with any clinical decisions regarding the treatment of the patients, and the treatment will adhere to the standard treatment at each trial site.

Patient and public involvement statement

The patients and the public were not involved in any phase of the trial (designing, recruiting or conducting). After completion of the trial, the media will be used for dissemination of the results.

Ethics and dissemination

Ethical considerations

The trial protocol has been reviewed and approved by the Regional Ethics Committee of the Capital Region (H-20056592) on 1 January 2021 and the Danish Medicines Agency (EudraCT 2020-002459-40) on 2 August 2020. The trial is monitored by the Good Clinical Practice units in Denmark.

There are currently no clinical guidelines in Denmark regarding the use of locally applied antibiotics in breast implantation, and the treatment depends on the individual surgeon's preference. Allocation to placebo in this trial is therefore considered ethically acceptable. A detailed description of the ethical considerations is provided in the supplemental material.

Safety considerations

Previous studies have shown that the serum level after local application of antibiotics is low,[27,28] and therefore the risk of systemic side effects is low. Gentamicin, vancomycin and cefazolin have been used for local application on breast implants for many years and are considered safe.[16,29] If the patients experience adverse events, it will be registered in an electronic case report form in

REDCap and treated according to local guidelines. All serious adverse reactions will be reported yearly to the Danish Medicines Agency and the Regional Ethics Committee by the sponsor-investigator during the study period. A more detailed description of the safety considerations is provided in the protocol in the supplemental material.

Consent

Consent from trial participants is obtained according to Danish legislation.[30] The investigators are responsible for obtaining the signed, informed consent from the patients prior to any protocol-related activities. The consent can be withdrawn by the patient at any time and without explanation, after which the patient will receive the standard treatment according to the local guidelines.

Dissemination and data sharing

The main paper will include the primary and secondary outcomes. The manuscript will adhere to the CONSORT guidelines and will be used to report the results of the trial to the scientific community. The manuscript will be submitted to an international peer-reviewed journal, and both positive, negative and inconclusive results will be published. The findings of the trial will be shared with participating sites and presented at national and international conferences. The results will be registered at ClinicalTrials.gov and will be disseminated to the public. After publication of the results, researchers and other relevant parties can be granted access to anonymized data upon request.

Status

The first patient was enrolled in the trial in January 2021, and the trial is currently recruiting. The last patient is expected to be included in January 2024. The primary results of the trial are anticipated in July 2024 after the last patient's last follow-up. The results from this trial can be used in evidence-based treatment guidelines for implant-based breast reconstruction surgery.

References

1. Eltahir Y, Werners LLCH, Dreise MM, et al. Quality-of-life outcomes between mastectomy alone and breast reconstruction: comparison of patient-reported BREAST-Q and other health-related quality-of-life measures. *Plast Reconstr Surg*. 2013;132(2):201e-209e.

2. American Society of Plastic Surgeons. *2019 Plastic Surgery Statistics Report*.; 2019. https://www.plasticsurgery.org/documents/News/Statistics/2019/plastic-surgery-statistics-full-report-2019.pdf.

3. Coroneos CJ, Selber JC, Offodile AC, Butler CE, Clemens MW. US FDA breast implant postapproval studies: long-term outcomes in 99,993 patients. *Ann Surg*. 2019;269(1):30-36.

4. Stevens WG, Calobrace MB, Alizadeh K, Zeidler KR, Harrington JL, d'Incelli RC. Ten-year Core Study Data for Sientra's Food and Drug Administration–Approved Round and Shaped Breast Implants with Cohesive Silicone Gel. *Plast Reconstr Surg*. 2018;141(4S):7S-19S.

5. Maxwell GP, Van Natta BW, Bengtson BP, Murphy DK. Ten-year results from the Natrelle 410 anatomical form-stable silicone breast implant core study. *Aesthetic Surg J*. 2015;35(2):145-155.

6. Petersen A, Eftekhari ALB, Damsgaard TE. Immediate breast reconstruction: a retrospective study with emphasis on complications and risk factors. *J Plast Surg Hand Surg*. 2012;46(5):344-348.

7. Dikmans REG, Negenborn VL, Bouman M-B, et al. Two-stage implant-based breast reconstruction compared with immediate one-stage implant-based breast reconstruction augmented with an acellular dermal matrix: an open-label, phase 4, multicentre, randomised, controlled trial. *Lancet Oncol*. 2017;18(2):251-258.

8. Pajkos A, Deva AK, Vickery K, Cope C, Chang L, Cossart YE. Detection of subclinical infection in significant breast implant capsules. *Plast Reconstr Surg*. 2003;111(5):1605-1611.

9. Rieger UM, Mesina J, Kalbermatten DF, et al. Bacterial biofilms and capsular contracture in patients with breast implants. *Br J Surg*. 2013;100(6):768-774.

10. Benediktsson K, Perbeck L. Capsular contracture around saline-filled and textured

subcutaneously-placed implants in irradiated and non-irradiated breast cancer patients: Five years of monitoring of a prospective trial. *J Plast Reconstr Aesthetic Surg*. 2006;59(1):27-34.

11. Calobrace MB, Stevens WG, Capizzi PJ, Cohen R, Godinez T, Beckstrand M. Risk factor analysis for capsular contracture: A 10-year sientra study using round, smooth, and textured implants for breast augmentation. *Plast Reconstr Surg*. 2018;141(4S):20S-28S.

12. Chopra K, Gowda AU, McNichols CHL, Brown EN, Slezak S, Rasko Y. Antimicrobial Prophylaxis Practice Patterns in Breast Augmentation: A National Survey of Current Practice. *Ann Plast Surg*. 2017;78(6):629-632.

13. Yalanis GC, Liu E-W, Cheng H-T. Efficacy and safety of povidone-iodine irrigation in reducing the risk of capsular contracture in aesthetic breast augmentation: a systematic review and meta-analysis. *Plast Reconstr Surg*. 2015;136(4):687-698.

14. Kenna DM, Irojah BB, Mudge KL, Eveler K. Absorbable antibiotic beads prophylaxis in immediate breast reconstruction. *Plast Reconstr Surg*. 2018;141(4):486e-492e.

15. Nava MB, Catanuto G, Rocco N. Reply: Role of Mitomycin C in Preventing Capsular Contracture in Implant-Based Reconstructive Breast Surgery: A Randomized Controlled Trial. *Plast Reconstr Surg*. 2017;140(6):823e-824e.

16. Hunsicker LM, Chavez-Abraham V, Berry C, McEwen D. Efficacy of vancomycin-based continuous triple antibiotic irrigation in immediate, implant-based breast reconstruction. *Plast Reconstr Surg - Glob Open*. 2017;5(12).

17. Tutela JP, Duncan DP, Kelishadi SS, Chowdhry S, Boyd T, Little JA. Continuous Postoperative Antibiotic Irrigation via Catheter System Following Immediate Breast Reconstruction. *Eplasty*. 2015;15:e49.

18. Hidalgo DA, Sinno S. Current trends and controversies in breast augmentation. *Plast Reconstr Surg*. 2016;137(4):1142-1150.

19. Lynch JM, Sebai ME, Rodriguez-Unda NA, Seal S, Rosson GD, Manahan MA. Breast pocket irrigation with antibiotic solution at implant insertion: a systematic review and meta-

analysis. *Aesthetic Plast Surg*. 2018;42(5):1179-1186.

20. National Institute for Health and Care Excellence (NICE). Surgical site infections: prevention and treatment. https://www.nice.org.uk/guidance/ng125/chapter/Recommendations#intraoperative-phase.

21. Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med*. 2013;158(3):200-207.

22. Guideline ICHHT. General considerations for clinical trials E8. In: *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use*. ; 1997.

23. Olsen MA, Nickel KB, Fox IK, Margenthaler JA, Wallace AE, Fraser VJ. Comparison of Wound Complications After Immediate, Delayed, and Secondary Breast Reconstruction Procedures. *JAMA Surg*. 2017;152(9):e172338.

24. TC H. Gaynes RP. Martone WJ. Jarvis WR. Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. *Infect Control Hosp Epidemiol*. 1992;13:606-608.

25. Pusic AL, Klassen AF, Scott AM, Klok JA, Cordeiro PG, Cano SJ. Development of a new patient-reported outcome measure for breast surgery: the BREAST-Q. *Plast Reconstr Surg*. 2009;124(2):345-353.

26. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *Trials*. 2010;11(1):32.

27. Sweet FA, Roh M, Sliva C. Intrawound application of vancomycin for prophylaxis in instrumented thoracolumbar fusions: efficacy, drug levels, and patient outcomes. *Spine (Phila Pa 1976)*. 2011;36(24):2084-2088.

28. White IV RR, Pitzer KD, Fader RC, Rajab MH, Song J. Pharmacokinetics of topical and intravenous cefazolin in patients with clean surgical wounds. *Plast Reconstr Surg*. 2008;122(6):1773-1779.

29. Campbell CA. The Role of Triple-Antibiotic Saline Irrigation in Breast Implant Surgery. *Ann Plast Surg*. 2018;80(6S Suppl 6):S398-S402.

30. Retsinformation. Lov om et videnskabsetisk komitesystem og behandling af biomedicinske forskningsprojekter. 30/05/2003. https://www.retsinformation.dk/eli/lta/2003/402. Published 2003. Accessed April 19, 2021.

Author contributions

All authors took part in designing the trial protocol and have contributed to the final manuscript. Mathilde N Hemmingsen, Andreas Larsen, Tim K Weltz and Anne K Bennedsen constitutes the trial coordinating unit who are responsible for coordinating all trial related activities. Mikkel Herly is the initiator and local site investigator at Rigshospitalet. Tine E Damsgaard is the holder of the grant from the Novo Nordisk Foundation and a member of the trial steering committee along with Søren J Sørensen, Thomas Bjarnsholt and Peter Vester-Glowinski. Camilla Bille, Lena F Carstensen, Lisbet Rosenkrantz Hölmich, Rikke Bredgaard, Lisa Toft Jensen, Vibeke Koudahl and Volker J Schmidt are local site investigators. Mathias Ørholt and Sebastian Wiberg are members of the blinded data assessment committee and provided statistical expertise.

Funding and sponsor

This work is supported by the Novo Nordisk Foundation (grant number 0058322) and the Medicine Fund of the Danish Regions (grant number R-189-A4127). The sponsor-investigator, Mikkel Herly, is the initiator of the trial.

Competing interests

None of the authors have any competing interests to declare.

Figure legends

Figure 1: Illustration of the trial intervention. The trial drug contains 1000 mg Vancomycin, 1000 mg Cefazolin and 80 mg Gentamicin dissolved in an infusion bag containing 500 ml of sterile isotonic saline. The placebo solution consists of 500 ml sterile isotonic saline.

Figure 2. Overview of the trial design. The patients are randomized to antibiotic treatment or placebo applied directly onto the breast implant and in the dissected tissue pocket. Patients who undergo bilateral breast reconstruction are randomized to antibiotics on one side and placebo on the contralateral side. Patients who undergo unilateral breast reconstruction are randomized to either antibiotics or placebo.

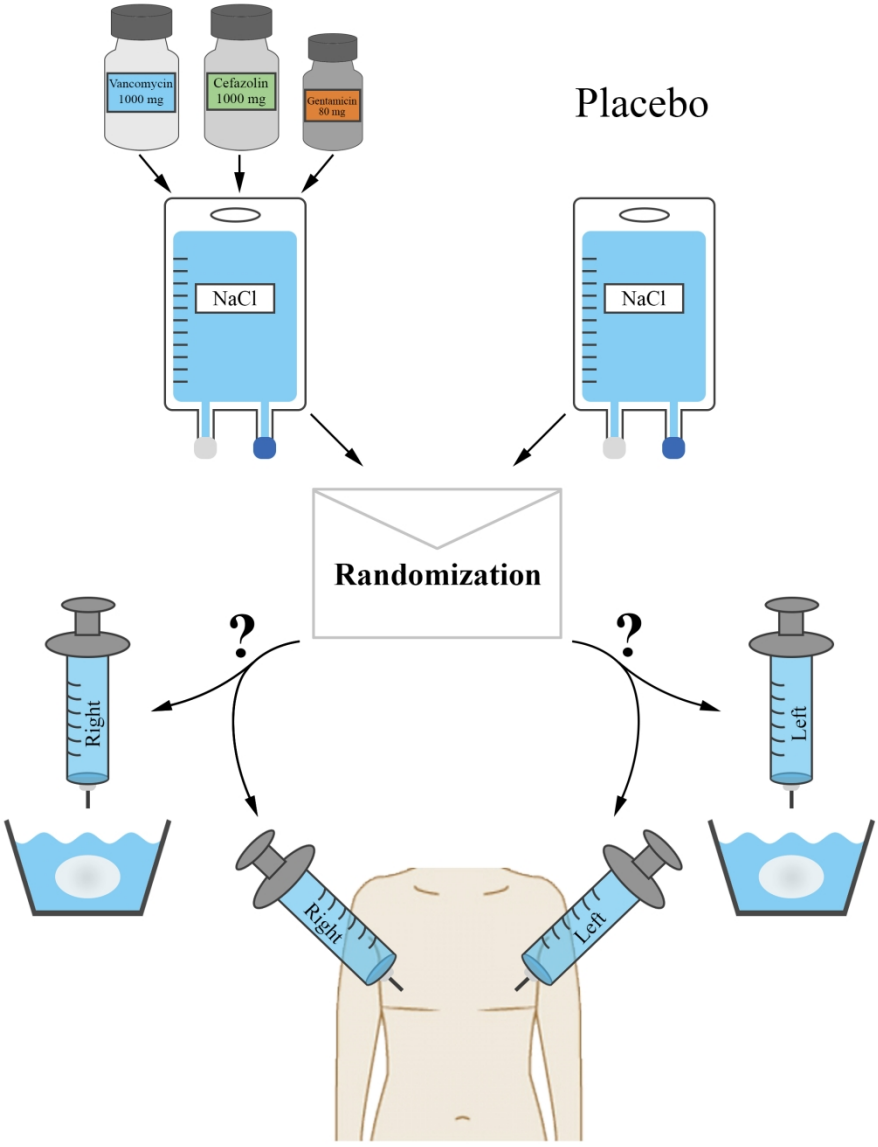


Figure 1: Illustration of the trial intervention. The trial drug contains 1000 mg Vancomycin, 1000 mg Cefazolin and 80 mg Gentamicin dissolved in an infusion bag containing 500 ml of sterile isotonic saline. The placebo solution consists of 500 ml sterile isotonic saline.

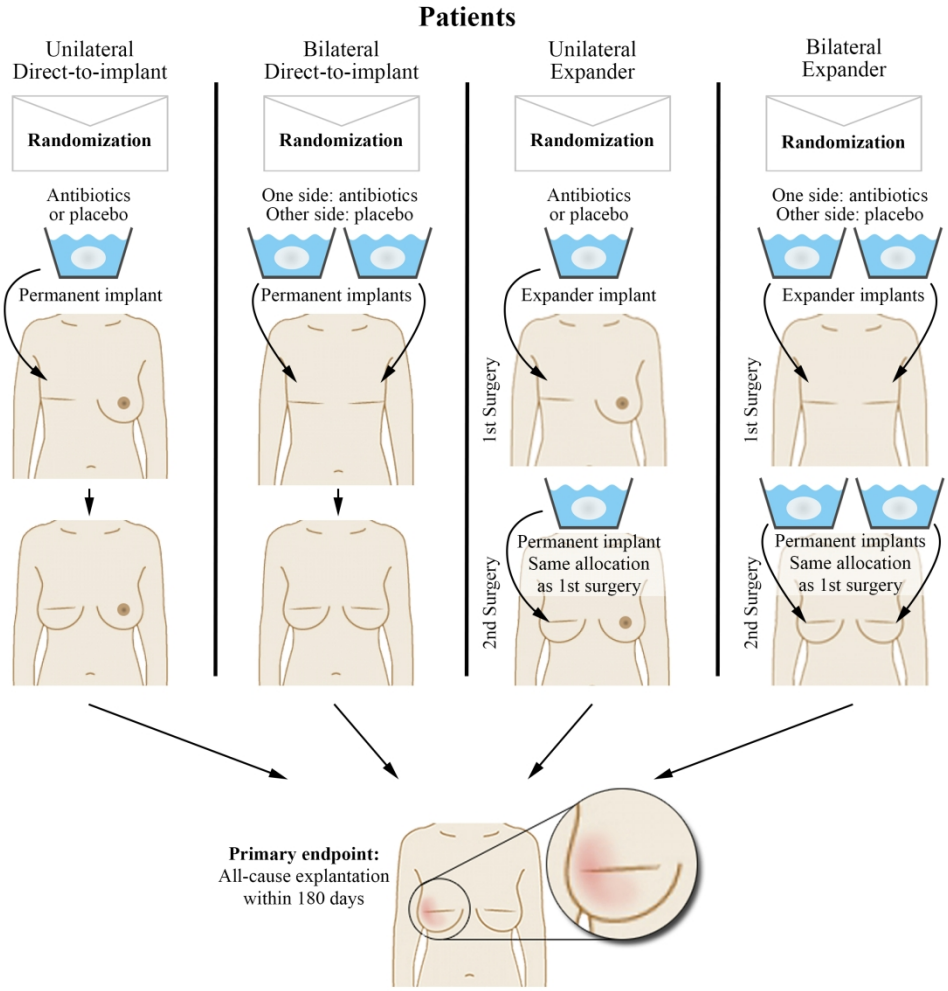


Figure 2. Overview of the trial design. The patients are randomized to antibiotic treatment or placebo applied directly onto the breast implant and in the dissected tissue pocket. Patients who undergo bilateral breast reconstruction are randomized to antibiotics on one side and placebo on the contralateral side. Patients who undergo unilateral breast reconstruction are randomized to either antibiotics or placebo.

Version 2.7
08/16/2021

Trial Protocol

Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Short Title: Local Antibiotics for Breast Implants

Acronym: The Breast-AB Trial

By

Mikkel Herly, MD
Mathilde Hemmingsen, MD
Andreas Larsen, MD
Tim Weltz, BMSc

Version: 2.7 (08/16/2021)

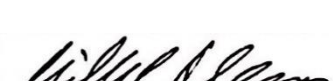
Sponsor Protocol Code Number: BREAST-AB-01

EudraCT number: 2020-002459-40

Date of Registration: 05-12-2020

Funding number: Novo Nordisk Foundation 0058322

Coordinating Sponsor-Investigator:
Mikkel Herly, MD
Department of Plastic Surgery and Burn Treatments
Blegdamsvej 9, 2100 Copenhagen
Phone: +45 35 45 33 53

Date: 08/16/2021 Signature: 

Version 2.7
08/16/2021

Trial synopsis

Title: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Lay title: Local Antibiotics for Women Undergoing Breast Reconstruction Surgery with Implants

Acronym: The BREAST-AB Trial

Trial design: A multi-center, investigator initiated, 1:1 randomized, double blind, placebo-controlled trial

Intervention: Application of gentamicin, vancomycin and cefazolin in a saline solution onto the implant and the dissected breast pocket used for breast reconstructive surgery

Objective: To determine the efficacy of local antibiotics in decreasing all-cause implant explantation

Inclusion criteria: Age ≥ 18, female, signed informed consent, breast reconstruction with implants including immediate/delayed reconstructions, bilateral/unilateral reconstructions and with or without flap reconstruction

Exclusion criteria: Pregnancy, breast feeding, known allergy towards any of the applied antibiotics, known anaphylactic reaction towards the same class of antibiotics as used in the trial, known allergy towards neomycin, known impaired renal function with GFR < 60 ml/min, participation in investigational drug trials and projects concerning disinfecting agents in the implant pocket and myasthenia gravis disease

Primary outcome: All-cause explantation of the breast implant within 180 days after the breast reconstruction surgery

Secondary outcomes:

- Time to explantation (days)
- Revision surgery with incision of the fibrous capsule within 180 days after the breast reconstruction surgery (Y/N)
- Exchange of permanent implant to expander implant within 180 days after the breast reconstruction surgery (Y/N)
- Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction surgery (Y/N)

Tertiary outcomes: Assessed for patients undergoing unilateral breast reconstruction

- Time from surgery to discharge (days)
- Re-admission within 180 days after the surgery (Y/N)

Long-term outcomes: All-cause incision of the fibrous capsule and capsular contracture after 5, 10 and 15 years

Sample size: A total number of 1274 breasts undergoing breast reconstruction will be included in the trial. Assuming that 27 % of the patients undergo bilateral breast reconstruction, this entails 1003 included patients

Trial duration: 3 years and 180 days

Randomization: Stratified randomization according to the following factors:

- Unilateral or bilateral reconstruction
- Immediate or delayed reconstruction
- Previous or scheduled radiotherapy within the follow-up period (yes/no)

All patients undergoing unilateral breast reconstruction will be randomized to the trial drug or placebo in a ratio of 1:1. All patients undergoing bilateral reconstruction will be randomized to the trial treatment on one of their breasts and placebo to the contralateral breast. Combining these factors gives a total of 14 randomization strata per trial site. An allocation sequence will be made for each stratum and assign treatment in a fixed block size of two to ensure that the investigational drug and placebo is evenly distributed within each stratum

Treatment: The intervention treatment will consist of 1000 mg vancomycin (bactocin), 2 mL of 40 mg/mL gentamicin (hexamycin) and 1000 mg cefazolin (cefazolin "MIP") in a 500 mL sterile isotonic (9 %) saline solution. The placebo solution will consist of 500 mL of sterile isotonic (9%) saline. During the surgery, the responsible nurse will draw three 50 ml syringes from the infusion bag (in total 150 ml) and use it to wash the dissected implant pocket. Another 50 ml syringe will be drawn from the same infusion bag and used to wash the implant with the assigned solution prior to insertion in the implant pocket

Clinical follow-up: The included patients will adhere to the standard follow-up program according to the guidelines of the local treatment site

Blinding: The patients, surgeons and data assessors will be blinded to the treatment allocation throughout the trial period. The coordinating sponsor-investigator will be responsible for monitoring adverse events. The trial coordinating unit will have access to the randomization sequences. They will not take part in any treatment of the participants or analysis of data. In the case of emergency unblinding the trial coordinating unit can always be contacted

Safety: Treatment-related adverse events will be reported and assessed continuously throughout the trial period

Trial Steering Committee

Thomas Bjarnsholt, Civil Engineer, DMSc, PhD
Professor at the Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet and Head of Costerton Biofilm Centre, Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

Tine Engberg Damsgaard, MD, PhD, MRBS
Professor at the Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet and at the Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Peter Viktor Vester-Glowinski, MD, PhD
Medical Doctor and post doc at the Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet

Mikkel Herly, MD, PhD
Medical Doctor at the Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet and associate professor at the Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

Søren J Sørensen, Cand. Scient., PhD
Professor and Head of the Section of Microbiology, Department of Biology, University of Copenhagen, Copenhagen, Denmark

Trial Coordinating Unit

Mathilde Nejrup Hemmingsen, MD
Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet

Andreas Larsen, MD
Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet

Tim Kongsmark Weltz, BMSc
Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet

Version 2.7
08/16/2021

Data Assessment Committee

Mathias Ørholt, MD
Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet

Sebastian Wiberg, MD, PhD
Department of Anesthesiology and Intensive Care, Zealand University Hospital, Køge

An independent biostatistician who will be appointed before the assessment committee receives the data

Version 2.7
08/16/2021

Site Investigators	Clinical Trial Site
Lena Felicia Carstensen, MD	Department of Plastic Surgery, South-West Jutland Hospital, Finsensgade 35, 6700 Esbjerg
Mikkel Herly, MD, PhD	Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen,
Lisbet Rosenkrantz Hölmich, Professor, MD, DMSc	Department of Plastic and Reconstructive Surgery, Copenhagen University Hospital, Herlev and Gentofte, Borgmester Ib Juuls Vej 1, 2730 Herlev,
Vibeke Koudahl, MD, PhD	Department of Plastic Surgery, Hospital Little Belt, Kabbeltoft 25, 7100 Vejle
Volker-Jürgen Schmidt, Ass. Professor, MD	Department of Plastic and Breast Surgery, Zealand University Hospital, Roskilde, Sygehusvej 10, 4000 Roskilde
Camilla Bille, MD, PhD	Department of Plastic Reconstructive Surgery, Odense University Hospital, J. B. Winsløws Vej 4, 5000 Odense

Pharmaceutical manufacturer

Vancomycin
Bactocin®
MIP Pharma GmbH
Kirkeler Strasse 41
D-66 440 Blieskastel
Tyskland
Tlf: (+49) 68 42 96 09 0
Fax: (+49) 68 42 96 09 35
Email: info@mip-pharma.de
Website: www.mip-pharma.de

Cefazolin
Cefazolin "MIP"
MIP Pharma GmbH
Kirkeler Strasse 41
D-66 440 Blieskastel
Tyskland
Tlf: (+49) 68 42 96 09 0
Fax: (+49) 68 42 96 09 35
Email: info@mip-pharma.de
Website: www.mip-pharma.de

Gentamicin
Hexamycin
Sandoz A/S
Edvard Thomsens Vej 14
2300 København S
Tlf: 63951000
Fax: 63951001
Email: info.danmark@sandoz.com
Website: www.sandoz.dk

Natriumklorid
Natriumklorid "Fresenius Kabi" 9 mg/ml
Fresenius Kabi Denmark
Islands Brygge 57
2300 København S
Tlf: 33181600
Fax: 33181614
Emailadresse: info-dk@fresenius-kabi.com
Website: www.fresenius-kabi.com

Version 2.7
08/16/2021

Table of contents

Version 2.7
08/16/2021

1. Introduction

The incidence of breast cancer in Danish women is approximately 4700 per year.[1] Many women choose to undergo breast reconstruction with an implant following a mastectomy.[2] Unfortunately, implant-based breast reconstructions are associated with high complication rates. Postoperative infection of the breast and implant is one of the most severe short-term complications affecting around 5-10 % of the women.[3–7] Clinically infected implants must be surgically removed and the recovery period that follows is long and agonizing for the women. Subsequent attempts to reconstruct the breast are often postponed for several months or abandoned altogether.

Many strategies to prevent complications associated with bacterial contamination of the breast implant have been attempted.[8–13] According to a survey made by the American Society of Plastic Surgeons, the most widely followed approach is to apply antibiotics directly on the breast implant and the dissected tissue pocket to eliminate bacterial contamination during the surgery.[14] Although the use of local antibiotics on breast implants is now widespread, the treatment regimen has never been investigated in a randomized controlled trial.[15] This protocol will describe a randomized controlled trial that will investigate the effect of antibiotics applied locally on the implant and in the breast implant pocket on the incidence of infection that leads to explantation of the implant. The protocol has been designed in accordance with the SPIRIT 2013 Statement guidelines for protocol content.[16]

1.1. Bacterial contamination of the breast implant

The most prevalent bacterial agents associated with breast implant infections are similar to those of the breast duct and skin flora which suggests that these are possible sources of contamination.[17] The most common microorganisms found on infected breast implants are *Staphylococcus epidermidis* and *Cutibacterium acnes* (previously known as *Propionibacterium acnes*). Other bacteria that have been identified on breast implants are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.[18,19]

Previous studies propose that bacterial colonization of breast implants sometimes occur without any immediate clinical manifestations.[20,21] Instead, bacteria form a chronic, subclinical infection which is suspected to play a pivotal role in the development of a protracted immune reaction to the implant known as capsular contracture, affecting approximately 10-20 % of the patients.[22,23]

1.2. Local administered antibiotics

Local administration of antibiotics can achieve a high local concentration with a low systemic uptake[24] and thereby, minimize the systemic side effects while achieving high antibiotic penetrance. A high local concentration can ensure optimal effect of the antibiotics at the surgical site,[25] and thereby decrease the rate of postoperative surgical site infections, while potentially minimize the risk of antibiotic resistance. Local antibiotics also have the benefit of being independent from the tissue vascularization to achieve peak concentration as opposed to systemic antibiotics, which is an advantage during larger surgeries where the vascularization can be compromised.[25]

Studies have shown that the concentration of locally applied antibiotics in the surgical drain output is high during the first 24 hours[24] and after 72 hours, the concentration is negligible. Therefore, it is assumed that the potential side effects to the medication will occur within the first 72 hours and previous studies have not reported side effects to the local treatment.[26]

In 2001, Adams et al recommended an antibiotic regimen consisting of gentamicin, cefazolin combined with either bacitracin or vancomycin.[27] Internationally, this irrigation regimen has become the most commonly used for local breast pocket and implant irrigation.[28] In this trial we will investigate the combinations of gentamicin, cefazolin and vancomycin for irrigation for the breast pocket and breast implant.

1.3. Pre-clinical data

Preclinical data from in vitro models suggest that the combination of Gentamicin, Cefazolin and Vancomycin is the most efficient treatment against the bacterial species most commonly associated with breast implants.[27,29] Animal studies suggest that the local application of these antibiotics is safe.[30–33]

1.4. Clinical data

Current Evidence – a systematic review

The regimen of local antibiotics for breast implants and the dissected implant pocket has been widely applied in humans[14], but few studies have investigated the clinical effect. In May 2020, we searched scientific literature databases including Embase, Cochrane, Pubmed and Web of Science. We used the following search terms (((breast) AND (implant OR expander OR augmentation OR reconstruction)) AND (irrigation OR antibiotics OR antibacterial OR antiinfective OR antimicrobial OR disinfection OR bacitracin OR gentamicin OR vancomycin OR cefazolin OR neomycin)) AND (infection OR "capsular contracture" OR "capsular contraction" OR capsulitis). We included studies and reviews investigating the effect of any local antibiotics for irrigation of the implant and/or implant pocket in women undergoing implant-based breast reconstruction or cosmetic breast augmentation. Studies that did not list outcomes that were relevant for the primary and secondary outcomes of the BREAST-AB trial were excluded. The search identified 1697 studies of which 17 studies were included after title/abstract and full text screening. Seven review articles,[15,34–39] two prospective studies[40,41] and eight retrospective studies[41–49] were identified. No randomized controlled trials were identified. Most of the included studies included solely reported on patients undergoing cosmetic augmentation. Two studies included patients undergoing cosmetic breast augmentation and breast reconstruction, but they did not stratify the outcome.[48,49]

Two studies found a significant decrease in the infection rate when applying local antibiotics compared to a control group,[42,43] whereas one study found no significant decrease.[44] These studies were limited by the relatively small study populations and a poorly defined outcome and none of the studies were blinded. The rate of capsular contracture was found to be significantly decreased in two studies,[44,45] two studies found no significant decrease,[43,46] whereas one study found a significant increase in the capsular contracture rate.[41] However, all studies investigating

capsular contracture were limited by a short follow-up period for this long-term outcome. No adverse events have been reported in any of the included studies, and the local antibiotics were generally considered well-tolerated. See appendix 1 for a table of characteristics of the included studies.

1.5. Rationale

Administration of antibiotics directly on to the breast implant and dissected implant pocket will give a high concentration of the antibiotics where they are needed which may prevent bacterial contamination of the implant. This may decrease the rate of postoperative infections that lead to explantation of the implant and thereby improve the outcome for the patients.

1.6. Hypothesis

Local administration of gentamicin, cefazolin and vancomycin on the breast implant will decrease the rate of postoperative clinical infections compared to placebo.

2. Experimental design

2.1. Trial design

This trial is an investigator-initiated, randomized, double-blind and placebo-controlled clinical phase III trial. The triple antibiotic solution or placebo solution will be applied directly onto the implant used for breast reconstruction and the implant pocket. The included subjects who undergo bilateral reconstruction will be randomized to the triple antibiotic solution to one of their breasts and placebo to the contralateral breast. Those who undergo unilateral reconstruction will be randomized to the triple antibiotic solution or the placebo solution. See 4.2 for a more detailed description of the randomization. The triple antibiotic solution will consist of 1 g Vancomycin, 1 g Cefazolin and 80 mg Gentamicin diluted in 500 mL of saline.[27] The placebo solution will consist of 500 mL of saline. See section 5 for more information on the trial treatment.

2.2. Outcomes

2.2.1. Primary outcome

The primary outcome will be all-cause explantation of the breast implant within 180 days after the breast reconstruction surgery.

Definition

All-cause explantation will be defined as explantation of the implant without insertion of new implant. This will not include replacement of an expander with a permanent implant and replacement of implants due to cosmetic revisions such as asymmetry and implant rotation.

Rationale

The rationale for applying local antibiotics is to decrease the risk of severe complications associated with the presence of bacteria such as deep surgical site infection that leads to explantation of the implant. Postoperative infection that leads to explantation of the implant will

sometimes occur simultaneously with other complications where the cause of explantation may be unclear. Therefore, all-cause explantation is a logical and meaningful primary outcome.

2.2.2. Secondary outcomes

The secondary outcomes will include:

- Time to explantation (days)
- Revision surgery with incision of the fibrous capsule within 180 days after the breast reconstruction surgery (Y/N)
- Exchange of permanent implant to expander implant within 180 days after the breast reconstruction surgery (Y/N)
- Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction surgery (Y/N)

Definition

Time to explantation will be defined as the amount of days between the breast reconstruction and the implant explantation surgery. The breast reconstruction surgery will be defined as the surgery where they received the allocated treatment. Surgical site infection will be defined according to the CDC classification of surgical site infetion[50] leading to antibiotic treatment with oral or intravenous antibiotics administered after the surgery.

Rationale

Time to explantation is important to determine the relation to the breast reconstruction surgery and the etiology of the event that leads to explantation of the implant. In some cases, the reconstructed breast may be upheld despite complications associated with bacteria by revisional surgery and therefore revisional surgery is an outcome of importance. In other cases, the reconstructed breast can be upheld by exchanging the permanent implant with an expander which slowly allow expansion and preserved vitality of the vulnerable skin flaps before inserting the permanent implant. Local antibiotics may decrease the incidence of postoperative surgical site infection requiring antibiotic treatment. Postoperative swelling and redness are to be expected after a larger surgery and can be difficult to distinguish from signs of infection. Therefore, surgical site infection that leads to antibiotic treatment is a logical outcome.

2.2.3. Tertiary outcomes

The tertiary outcomes will be assessed for patients undergoing unilateral breast reconstruction. The tertiary outcomes will include:

- Time from the breast reconstruction surgery to discharge (days)
- Re-admission within 180 days after the breast reconstruction surgery (Y/N)

Definition

Time to discharge will be defined as the amount of days between the breast reconstruction and the day of discharge. The breast reconstruction surgery will be defined as the surgery where the patient received the allocated treatment.

Rationale

The rationale for excluding bilateral patients from the tertiary outcomes is that all bilateral patients will receive placebo on one breast and the intervention on the contralateral breast. Therefore, patient related outcomes are only applicable for patients who undergo unilateral breast reconstruction. Postoperative infection that occurs during the hospital admission can prolong the admission period. Application of local antibiotics may shorten the admission period by decreasing the rate of postoperative infection occurring during the hospital admission. Infection that occurs after discharge can cause re-admission to the hospital. Local antibiotics may decrease the infection rate after discharge that require hospitalization.

2.2.3 Additional follow-up

The trial will include additional long-term outcomes focused on all-cause incision of the fibrous capsule around the breast implant, capsular contracture, Baker classification[51] and quality-of-life. See Gantt chart figure 1.

Definition

Capsular contracture and the Baker classification grade will be obtained from the National Patient Registry and the patients' medical journals after 5, 10 and 15 years. The BREAST-Q questionnaire[52] will be used to assess patient-reported outcomes. The patients will be contacted and asked to fill out the questionnaire with 5 year-intervals after the surgery.

Rationale

Previous studies suggest that bacterial contamination of the breast implant can occur without immediate clinical manifestation.[20,21] Instead, the bacteria form a chronic, subclinical infection which is suspected to play a pivotal role in the development of a protracted immune reaction to the implant called capsular contracture, affection 10-20 % of the patients.[22,23] The use of local antibiotics could potentially decrease the rate of capsular contracture by minimizing the bacterial contamination of the implant. Therefore, capsular contracture is a meaningful long-term outcome.

The application of local antibiotics may decrease the risk of postoperative complications and thereby decrease the risk of undergoing revision surgery. This in turn may lead to improved patient satisfaction and quality of life. Breast-Q is a validated tool used to quantify patient satisfaction and health-related quality of life after breast reconstruction surgery.[52]

Patients may be included in additional exploratory substudies at the time of implant explantation (e.g. expander removal). The exploratory substudies will be applied for in separate protocols to be approved by the relevant authorities and they will not interfere with this trial.

2.3. Setting and locations

The trial will be a nationwide multi-center trial with enrollment of patients from the following Danish hospitals:

- Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen
- Department of Plastic Surgery, Herlev and Gentofte Hospital, Borgmester Ib Juuls Vej 1, 2730 Herlev
- Department of Plastic Surgery, Zealand University Hospital, Sygehusvej 10, 4000 Roskilde
- Department of Plastic Surgery, Odense University hospital, J. B. Winsløws Vej 4, 5000 Odense
- Department of Plastic Surgery, South-West Jutland Hospital, Finsensgade 35, 6700 Esbjerg
- Department of Plastic Surgery, Hospital Little Belt, Kabbeltoft 25, 7100 Vejle

All sites have clinical experience and expertise in performing implant-based breast reconstructions.

2.4. Number of Subjects

The trial will include patients until a total number of 1274 breast reconstructions according to our power calculation. We estimate that this number will be distributed on approximately 1003 patients provided that approximately 27% of patients undergo bilateral procedures. A total of 637 breasts will be allocated to placebo and 637 breasts will be allocated to treatment with the local antibiotic solution. The statistical considerations behind the sample size calculation is elaborated in section 8.1.

2.5. Trial Duration

We plan to begin inclusion in January 2021 at Rigshospitalet and Herlev Hospital. The other trial sites will begin enrollment thereafter according to the plan outlined below. We expect to begin inclusion at Zealand- and Odense University Hospital in spring 2021 followed by South-West Jutland Hospital and Hospital Little Belt in the autumn 2021. Additional trial sites may be applied for during the trial period if we do not meet our expected aim for included patients. See Gantt chart in figure 1.

We expect to include the 1003 patients over a 3-year period with planned completion January 2024, hence the last 180-day follow-up will be completed after 3 years and 6 months. Currently, 700 women undergo reconstruction with implants each year in Denmark.[53] Therefore, we assume that it will be feasible to include approximately 334 patients per year.

Trial site	Inclusion period	Expected no. of included patients
Rigshospitalet	Jan 2021 – Jan 2024	274

Herlev Hospital	Jan 2021 – Jan 2024	253
University Hospital Zealand	Marts 2021 – Jan 2024	162
Odense University Hospital	Marts 2021 – Jan 2024	162
South-West Jutland Hospital	Sep 2021 – Jan 2024	76
Hospital Little Belt	Sep 2021 – Jan 2024	76
Total		**1003**

3. Subjects eligibility

All trial candidates will be evaluated for suitability by a medical doctor with expertise in the field of breast reconstruction surgery. All potential participating patients will receive oral information by the medical doctor and all information material will be given to the patient before the written informed consent form is signed. See participant timeline in figure 2.

3.1. Inclusion criteria

The patients must fulfill all the following criteria to be eligible for inclusion in the trial:

- Age ≥ 18 years
- Biologically female
- Signed informed consent
- Scheduled for breast reconstruction with implants or expanders including:
    a. Immediate or delayed reconstructions
    b. Bilateral or unilateral reconstructions
    c. With or without simultaneous flap reconstruction

3.2. Exclusion criteria

Patients are considered ineligible if any of the following criteria is fulfilled:

- Pregnancy
- Breast feeding
- Known allergy towards Vancomycin, Gentamicin and Cefazolin
- Known anaphylactic reaction towards other beta-lactam antibiotics or aminoglycosides
- Known allergy towards neomycin
- Known impaired renal function with GFR < 60 mL/min
- Participation in investigational drug trials and projects concerning disinfecting agents in the breast implant cavity
- Myasthenia Gravis

3.3. Pregnancy

Fertile women with child-bearing potential must provide a negative urine HCG prior to inclusion in the trial.

4. Enrollment

The patients will be registered in the trial after providing written consent (via the written consent form or a digital signature). The registered patients are considered enrolled in the trial when they have received the treatment. Each step of the enrollment procedure is described below.

4.1. Registration

All patients scheduled for a preoperative visit concerning a breast reconstruction procedure will be screened for eligibility and recorded in the individual trial site's screening log (appendix 2). No personal data will be recorded in the screening log. The following variables will be registered in the screening: screening number, screening date, initials of the person conducting the screening, age of the patient, if available date of pre-operative visit and surgery, eligibility of the patient yes/no and if "no", reason for non-eligibility. All patients who are considered eligible and have provided a written consent will be registered in the trial with a letter code for each site (e.g. RH for Rigshospitalet) combined with a record ID. The record ID will be assigned in sequential order as subjects are registered (1, 2, 3). Registration will include date of registration, central registration number, unilateral or bilateral reconstruction, type of surgery (immediate or delayed reconstruction) and radiotherapy status. The identification number remains constant throughout the trial.

4.2. Randomization and treatment assignment

Registered subjects will be randomized to placebo or the trial drugs on the day of surgery or the day before, and they will be considered enrolled in the trial when they have received the trial treatment. The randomization number will be the same as the record identification number. All patients undergoing unilateral breast reconstruction will be randomized to the trial drug or placebo in a ratio of 1:1. All patients undergoing bilateral reconstruction will be randomized to the trial treatment on one of their breasts and placebo to the contralateral breast (Investigational Product Dosage and Administration, section 5). See figure 3.

We will use a stratified randomization to ensure that potential risk factors which could confound the outcome are evenly distributed in the placebo and intervention group. The randomization strata will be generated by the following factors:

- Unilateral or bilateral reconstruction
- Immediate or delayed reconstruction
- Previous radiotherapy and/or planned radiotherapy within the follow-up period (yes/no)

When the three factors are combined, we get a total of 14 randomization strata per trial site. See appendix 3 for an overview of the 14 randomization strata. An allocation sequence will be made for each stratum and assign treatment in a fixed block size of two to ensure that the investigational drug and placebo is evenly distributed within each stratum. The fixed block size of two will not increase the risk of the investigator anticipating the allocation because the investigators are blinded to the treatment throughout the trial.

A member of the trial coordinating unit will access the computer-generated allocation sequence via RedCap The allocation will be registered in a REDCap module only available for members of the trial coordinating unit, who are unblinded.

4.2.1. Treatment assignment in two-stage breast reconstruction

Most implant-based breast reconstructions are performed in a single surgery with a permanent breast implant. During the surgery, the surgeon evaluates the quality and vitality of the dissected skin flaps before inserting the breast implant. In some patients, the skin quality is not considered suitable to allow for insertion of the permanent implant. These patients will be reconstructed in two stages, where an expander implant is used in the first surgery. The expander implant is used to expand the tissue before it can be replaced with a permanent implant after approximately 3 to 9 months of expansion. During the first surgery where the expander implant is inserted, the patient will be assigned to treatment according the randomization stratum. The allocation sequence number will be registered. At the second surgery where the expander is replaced with the permanent implant, the same treatment allocation will be used (e.g., if the right breast received treatment with the antibiotic solution in the first surgery, the right breast will receive treatment with the antibiotic solution in the second surgery). See figure 3.

4.2.2. Treatment assignment if a unilateral patient later becomes bilateral

In some cases, a unilateral patient can switch to the bilateral set-up. An example could be that a patient develops unilateral breast cancer, undergo mastectomy and reconstruction and then later in the trial period decide to undergo prophylactic risk-reducing mastectomy and reconstruction of the other breast. In this case, the patient would be assigned to either placebo or the trial drug in the first surgery. Then, when the patient undergo the second prophylactic surgery on the other breast, she will be allocated to receive placebo, if she had received antibiotics in the first surgery and vice versa, if she had received placebo in the first surgery she would be allocated to antibiotics on the other breast in the second surgery.

4.3. Registration failures

Registered subjects who are ineligible for randomization will be recorded as screening failures and they will be registered along with the reason for exclusion.

4.4. Discontinuation from the trial

Patients who withdraw their informed consent at any point during the trial period will be omitted from the trial and registered as “withdrawal of consent”. The coordinating sponsor investigator may omit participants from the trial at any point during the trial period due to safety of the participant. There will be no additional follow-up or data collection from these patients. The trial treatment is administered as a single dosage and therefore, exclusion will not have any effect on the trial treatment. The data analysis in the end of the trial will be performed on a modified intention-to-treat population, defined as all randomized patients with a valid informed consent.

4.4.1. Registration of dropouts

A designated representative at each trial site will be responsible for contacting the trial coordinating unit in case an included subject withdraws consent or is unable to complete the follow-up. All excluded patients will be recorded including date, central registration number, reason for exclusion and treatment allocation. Patients excluded from the trial will continue to follow the scheduled follow-up visits as a part of the standard treatment. Exclusion from the trial will not interfere with the standard treatment or entail any additional procedures or follow-up visits. Dropout rates will be monitored continuously by the sponsor-investigator.

4.4.2. Replacement of dropouts

Patients who drop out of the trial after enrollment (allocation to trial treatment) will not be replaced. The sample size calculation accounts for a drop-out rate of 5 %. In case of a drop-out rate of more than 5 %, we will apply imputations by chained equations and repeat the primary analysis (for the primary outcome) after imputations.

5. Treatment procedures

The trial drug will be administered as a single dosage during the breast reconstructive surgery. The administration procedure will be identical for the antibiotic solution and placebo. Only qualified healthcare personnel will perform the administration of trial drugs and no self-administration will take place. All personnel that handles investigational products will be instructed by members of the trial coordinating unit.

5.1. Investigational drugs

Gentamicin: 40 mg/ml gentamicin sulfate, 2 mL suspension in glass ampoules containing clear, colorless suspension without visible particles. Gentamicin is a broad-spectrum, bactericidal aminoglycoside primarily targeting gram-negative rods. Gentamicin (Hexamycin) produced by Sandoz A/S can be used but other producers of the same drug may be used as an alternative.

Cefazolin: 2096,72 mg cefazolin natrium equivalent to 2000 mg cefazolin of white or almost white powder in a capped vial. Cefazolin is a bactericidal antibiotic targeting both gram-negative and gram-positive bacteria. Cefazolin produced by MIP Pharma GmbH can be used but other producers of the same drug may be used as an alternative.

Vancomycin: one capped vial contains vancomycin hydrochloride equivalent to 1000 mg vancomycin as a finely grounded white powder with a pink to brown nuance. Vancomycin is bactericidal and targets gram-positive bacteria. Vancomycin (Bactocin®) produced by MIP Pharma GmbH can be used but other producers of the same drug may be used as an alternative.

All investigational drugs will be purchased through each trial site's clinical pharmaceutical services and their respective purchasing agreements. The investigational drugs will be kept and prepared in accordance with the manufacturer's recommendations (see 'summery of product characteristics' for cefazolin, gentamicin and vancomycin. The investigational drugs are part of the standard drug selection available at all trial sites. Therefore, we will not account for the overall stock of medicine.

However, we will account for the use of investigational medicine for each patient enrolled in the trial, including batch-number, expiration date, patient registration number and date of administration.

5.2. Preparation of the drug solutions

The preparation of the trial drug solution will take place in a medication room on the trial site on the day of the surgery or day before the surgery. A designated trained nurse will be responsible for the preparation and labelling of the trial drug solution. To minimize errors, it will be double checked. The labelling will include the trial identification number and marking of which breast the treatment will be applied to (right or left). The manufacturing nurse will contact the trial coordinating unit to confirm his/her identity as investigational drug manufacturer and obtain instruction as to how to allocate the trial treatment. The communication between the Trial coordinating unit and the manufacturing nurse will be looped to prevent mistakes regarding the allocation. A designated person will deliver the prepared solutions to the operation room, and the surgeon and the scrub nurse will be blinded for the allocation. A local SOP describing the preparation of the trial drugs will be compiled for each trial site (See appendix 4 for the SOP).

5.2.1. The antibiotic solution

The antibiotic solution will contain 1000 mg vancomycin, 80 mg gentamicin and 1000 mg cefazolin. Two syringes of 20 mL sterile saline will be drawn from an infusion bag containing 500 mL of sterile isotonic (9%) saline. The drawn saline will be infused in the capped vials containing cefazolin and vancomycin to dissolve the powder. The entire content of the vial containing vancomycin (20 mL) will be drawn back into the syringe and reinfused in the infusion bag. Only 10 mL of the cefazolin solution will be drawn from the capped vial containing the dissolved cefazolin. The 10 mL will also be reinfused in the infusion bag. The remaining content the capped vial will be discarded as medical waste. Hereafter, the 2 mL gentamicin suspension will be drawn into a syringe and infused in the infusion bag already containing vancomycin and cefazolin. See figure 4 for an illustration of the treatment preparation.

5.2.2. The placebo solution

The placebo solution will consist of 500 mL of sterile isotonic (9%) saline contained in a similar infusion bag.

Both the antibiotic solution and the placebo solution will be achromatic and will be indistinguishable from one another to ensure blinding of the health care personnel administering the drugs.

5.3. Investigational product administration

The assigned solution will be administered in an enclosed infusion bag. During the surgery, the responsible nurse will draw three 50 ml syringes from the infusion bag (entailing 150 ml) and use it to wash the dissected implant pocket. Another 50 ml will be drawn from the same infusion bag and used to wash the implant with the assigned solution prior to insertion in the implant pocket. The rest of the content in the infusion bag will be discarded as medical waste. Patients undergoing unilateral breast reconstruction will receive the assigned solution in one infusion bag marked either left or right. The patients undergoing bilateral breast reconstruction will be assigned to the

antibiotic solution to one breast and placebo solution to the contralateral breast, and the allocation sequence will determine which breast (right or left) gets which solution. The assigned solution bags will be marked right and left. See appendix 5 for SOP.

5.4. Dosage adjustments

The dose of investigational products will be fixed. The drug will be administered as a single dose, and one time only. No continuous administration will occur. It will not be possible to adjust the treatment after the administration of the treatment.

5.5. Concurrent medication

All included patients will be treated according to the local guidelines for breast reconstruction surgery at each trial site. The trial intervention will not interfere with any treatment procedures or administration of medication.

5.6. Blinding

The trial will double-blind so that the patients, site investigators and data monitors will be blinded to the allocation. Only the designated nurses and the members of the trial coordinating unit (who provide the randomization number and treatment allocation) are not blinded to the allocation. The unblinded persons will not in any way be part of the treatment, clinical evaluation of outcomes or data assessment.

The intervention drug will be prepared in infusion bags that will be indistinguishable to the placebo solution (infusion bags with saline). Both solutions are colorless and identical in appearance without any identifying features, and therefore we do not anticipate any risk of unintentional unblinding. The designated manufacturing nurse will contact the trial coordinating unit to receive the allocation by telephone. The randomization number and the treatment allocation will be provided by the trial coordinating unit. An emergency telephone number to the trial coordinating unit will be available to access the treatment allocation of individual patients in the case where emergency unblinding is necessary. If unblinding should occur, it will be documented via the case report form. The unblinded patient will not be excluded from the trial.

The patients will remain blinded until the end of the additional follow-up period. The rationale for keeping the patients blinded is to minimize bias when assessing the long-term outcomes. The patients can be unblinded upon request if they withdraw their consent to participate in the trial.

5.6.1. Ensuring blinding

The randomization number will ensure that the allocation is given during both surgeries in situations where the intervention treatment is repeated, for instance in two stage breast reconstruction (see section 4.2.1.) and if a unilateral patient becomes bilateral (see section 4.2.2.). During the first surgery, the registration number which equals the randomization number will be registered in the case report form. During the second surgery, the manufacturing nurse will contact the trial coordinating unit to obtain the treatment allocation and members of the trial

coordinating unit (who are unblinded) will look up the patient and inform the manufacturing nurse of the treatment allocation. The manufacturing nurse will not take part in any treatment procedures.

6. Data collection

The site investigators, along with members of the trial coordination unit, will be responsible for trial related data collection and entry. The individual trial site investigator may delegate assignments to designated doctors, scholarship students or nurses registered in the site-specific delegation log. Limited trial specific data will be entered into a numbered case report form (see appendix 6) at the time of inclusion and the surgery. This along with the screening log will be the only source data and all additional data will be obtained from the patients' medical records. Data will be entered directly into REDCap.

6.1. Variables

All enrolled patients (i.e. patients who have been assigned to treatment) will be entered into the database. An overview of included variables is provided below.

6.1.1. Pre-surgery variables

Trial related variables

- Study ID
- Site (location)
- Unilateral or bilateral breast reconstruction
- Immediate or delayed breast reconstruction
- Prophylactic or cancer (including carcinoma in situ)
- Radiation therapy status (Y/N)
- Name
- CPR number
- Date and name of data collector

Patient demographics

- Height (cm)
- Weight (kg)
- Smoking (never, former, active)
- Alcohol consumption (units per week)
- ASA classification (class I-VI)
- Race

Comorbidities

Prescription medications

Oral or intravenous antibiotic treatment within 2 months up to the surgery (Y/N, name and dose of antibiotic)

Prior breast surgery (Y/N, type of surgery, date)

Radiation therapy (dose and fraction)

Chemotherapy (type, dose, duration, frequency and no of cycles)

Antihormonal therapy (type, dose and duration)

Antibody therapy (type, dose and duration)

6.1.2. Surgical variables

Trial related variables

Date and time of surgery

Randomization number

Direct-to-implant or expander breast reconstruction

Deviations from the protocol

Date and time of treatment administration

Date and name of the data collector

Surgery characteristics

Mesh (Y/N)

Drain (Y/N)

Operative time (hours, minutes)

Implant type (brand, volume, texture, serial no.)

Implant placement (prepectoral or subpectoral)

Type of mastectomy procedure (nipple sparring KHAC10 or skin sparring KHAC15)

Type of reconstruction (KHAE00, KHAE05)

Thickness of mastectomy flap (mm)

Pre- and perioperative medications

VAC (Y/N)

6.1.3. Post-surgery variables

Characteristics

- Date of discharge
- Time to drain removal
- Post-operative medication
- Hematoma (Y/N)
- Mastectomy flap necrosis (Y/N)
- Nipple-areola-complex necrosis (Y/N)
- Seroma (Y/N)
- Explantation (Y/N)
- Date of explantation
- Indication of explantation
- Surgical site infection (Y/N)
- Bacterial agent (culturing/PCR)
- Revisional surgery with incision of the fibrous capsule (Y/N)
- Date of revisional surgery
- Indication of revisional surgery
- Baker grading
- Local adverse event
  - Severity of event
  - Time of event
  - Treatment/action taken
- Exclusion/loss to follow-up
  - Reason for exclusion

6.2. Clinical follow-up

The included patients will adhere to the standard follow-up program according to the guidelines of the local treatment site. There will no trial specific clinical follow-up visits. The patients will be instructed to contact the local treatment site if they should experience adverse events after they have been discharged. Additionally, the patients will be instructed to contact us if they receive relevant treatment related to the reconstructed breast at another hospital than their primary treatment site or via their general practitioner within 180 days after the surgery.

6.3. Data quality and security

Each variable is clearly defined in the case report form. Each data field will be provided with a definition of the variable, category for categorial variables and units for continuous variables.

All relevant trial documents including the signed consent form for each patient will be stored in the trial master file in a secure, locked place at each individual site. Only the site investigator and designated personnel will have access. The trial has been approved by the Danish Data Protection Agency. The files will be stored for 25 years, after which they will be destroyed.

7. Assessments of safety and harm

Women receiving breast cancer treatment and subsequently undergo breast reconstructive surgery are in high risk of experiencing adverse events in relation with the surgical treatment and cancer. These adverse events will not be registered as trial drug-adverse events.

The trial drugs are widely used internationally, and the adverse reaction profile for each drug is well-defined. (See 'Summery of Product Characteristics' section 4.8 for each drug). Previous studies show, that only low levels of locally administered antibiotics enter the bloodstream and therefore, the systemic side effects to the trial drugs are expected to be negligible.[54]

7.1. Expected adverse events unrelated to the trial drug

The complication rate following breast reconstruction is relatively high, and certain adverse events are to be expected after surgical resection of the breast and reconstruction with an implant. Several postoperative adverse events are likely to occur in the included patients due to the surgery and patient comorbidities and these events will not be registered as adverse events related to the interventional drugs. The following adverse events are expected in the included patients and will not be registered as adverse events to the trial treatment:

- Surgery specific complications: surgical site infection, implant malposition, expander deflation, expander port malfunction, implant/expander exposure, implant/expander rupture, wound dehiscence, hematoma, flap necrosis, seroma, capsular contracture, nerve damage, pain and lymphedema.
- Infectious disease including sepsis without signs of surgical site infection and fever without signs of surgical site infection.
- Cardiovascular disease including stroke, acute myocardial infarction, heart failure, arrythmia, cardiac arrest, pulmonary embolism, DVT and coagulopathy.
- Gastrointestinal disease including diarrhea, nausea, vomiting, gastroenteritis, colitis and ileus.
- Adverse events related to the kidneys and urinary tract including uremia, proteinuria, hematuria, interstitial nephritis, upper and lower urinary tract infection and pre-renal and post-renal kidney failure.
- Liver- and biliary tract disease including increase in serum liver enzymes levels, increase in bilirubin and alkaline phosphatase, hepatitis, liver cirrhosis, cholecystitis and pancreatitis.

- Respiratory disease including dyspnea, pleural effusion, pneumonia, pharyngitis, sinusitis and rhinosinusitis.
- Neurological disease including seizures, dizziness, impaired vision and vertigo.
- Metabolic disease including hyperglycemia and hypoglycemia.
- Immunological disease including thrombocytopenia, leukopenia, granulocytopenia, agranulocytosis, pancytopenia, leukocytosis, granulocytosis, anemia and polycythemia.
- Psychiatric disease including depression.
- Cancer recurrence.

7.2. Adverse events possibly related to the trial drug

The presumed relation to the trial drug will be evaluated using the 'Summery of Product Characteristics' for Gentamicin, Cefazolin and Vancomycin.

Examples of events that are likely to be related to the trial drugs are:

Erythema multiforme

Urticaria

Angioneurotic edema

Toxic epidermal necrolysis

Steven Johnsons' syndrome

Anaphylactic shock

Red man syndrome (appearing after a maximum of 10 minutes after administration)

Acute tinnitus

Acute deafness

Drug induced-acute kidney injury

Myasthenia gravis-like syndrome

Local adverse events (incl. surgical site infection, skin irritation, erythema, delayed wound healing, itching)

7.3. Adverse Event Reporting

Local antibiotics therapy is considered safe.[24] Previous studies have shown that the serum level of antibiotics after local application is low,[26] and therefore the risk of systemic organ toxicity is low. Gentamicin, cefazolin and vancomycin have all been approved for marketing for many years and have a well-known systemic adverse reaction profile. The combination of gentamicin, vancomycin and cefazolin has been used for local breast pocket irrigation for many years and is considered

safe.[55] Due to the extremely low systemic uptake when using locally administered antibiotics, the event of systemic adverse events following the trial intervention is considered very unlikely.

Adverse events related to the study drug will be assessed continuously by the co-investigator at each study site during the admission period (typically 72 hours). The trial drugs have a short half-life period of maximum 6 hours and will only be administered one time during the surgery. Therefore, it is considered very unlikely that adverse events related to the trial drugs should occur after discharge. If the patients experience adverse events, the site investigator will be responsible for registering the adverse event directly in the case report form. The patients will be instructed to contact the local treatment site if they should experience adverse events between the scheduled follow-up visits. The site investigator will report all adverse events to the coordinating sponsor-investigator who will be responsible for monitoring the safety of the trial. Each adverse event will require the investigator to fill in the AE form including the following variables: patient identification number, description of event, onset and end of event, severity, relation to the intervention, action taken and outcome.

Any adverse events occurring during the trial will be treated and monitored according to the local guidelines.

7.3.1. Adverse Events (AE) and Adverse Reactions (AR)

Any event that occurs after administrations of the trial drug regardless of the relation to the trial drug will be defined as an adverse event. Adverse reactions will be defined as events that are related to the trial drug.

7.3.2. Serious Adverse Events (SAE) and Serious Adverse Reactions (SAR)

For each recording of adverse event, the event will be evaluated as to whether it was a serious adverse event. A SAE will be defined as an AE that is life threatening, results in death, requires prolonged hospitalization or results in significant disability.

The site investigator at each trial site will be responsible for contacting the coordinating sponsor-investigator in case of serious adverse events within 24 hours of awareness. The site investigator will record the event in the case report form.

All serious adverse reactions will be reported yearly to the Danish Medicines Agency and the Regional Ethics Committee by the sponsor-investigator during the study period of three years and 6 months. After last patient last visit, serious adverse events will no longer be reported annually to the Danish Medicines Agency and The Regional Ethics Committee, since the effect of the study drug is considered negligible after 30 hours, and it is unlikely that any serious adverse events related to the study drug would occur after three days.

During the long-term follow-up, serious adverse events will not be reported in the annual safety report (ASR). Serious adverse events will be registered in the trial and included in a final clinical study report after the last long-term follow-up. See Gantt chart figure 1.

7.3.3. Suspected Unexpected Serious Adverse Reactions (SUSARs)

A SUSAR is an unexpected and serious presumed reaction to the trial drug. Section 4.8 in the 'Summery of Product Characteristics' for each drug (cefazolin, gentamicin and vancomycin) will be used as the reference safety information to determine whether or not the serious adverse event is unexpected. The sponsor-investigator will be responsible for that all relevant information about SUSARS, which are fatal or life threatening, is recorded and reported to the Regional Health Ethics Committee and the Danish Health and Medicines Authority as soon as possible, and no later than 7 days after the sponsor-investigator has been informed of such an event. No later than 8 days after the reporting, the sponsor-investigator is responsible for informing the Regional Health Ethics Committee and the Danish Medicines Agency of relevant treatment initiated by the co-investigator or a doctor at the trial site. Any other SUSAR must be reported to the Regional Health Ethics Committee and the Danish Medicines Agency no longer than 15 days after the sponsor-investigator has been informed. All trial investigators will be informed by the coordinating sponsor-investigator in the event of a SUSAR. See Gantt chart figure 1.

7.3.4. Severity of Adverse Events

The severity of each adverse event suspected to be related to the trial drug will be graded accordingly

- Mild: transient symptoms, with no interference in normal daily activity
- Moderate: persistent symptoms, resulting in moderate inhibition of daily activity
- Severe: persistent symptoms, resulting in severe inhibition of daily activity

7.3.5. Relationship of AE to Trial Intervention

For each AE suspected to be related to the trial drug, the probability will be rated accordingly

- Probable: there is good reason and adequate documentation to assume causal relationship
- Possible: a causal relationship is likely and cannot be dismissed
- Unlikely: the event is most likely related to an etiology other than the intervention
- Unknown: causality is not assessable

7.3.6. Adverse Reactions reporting during long-term follow-up

The annual reporting of SAR will not apply during the long-term follow-up period. Serious adverse reactions that we learn of during the long-term follow-up period of 5, 10 and 15 years will be recorded and included in a final clinical study report after the last long-term follow up.

SUSARS will be reported continuously to the Danish Medical Agency throughout the long-term follow-up period. See Gantt chart in figure 1.

8. Statistical considerations

8.1. Sample size

The trial will be powered towards the primary endpoint. Previous data[3] do not suggest that there is an overrepresentation of bilateral infections compared to the unilateral infection rate which suggests that infections may be randomly distributed. Therefore, we do not assume that infections are correlated between breasts within the same patient. However, due to the paired design in the patients that undergo bilateral reconstruction, any correlation between the individual patient's breasts will increase the statistical power of this trial. The independent sampling unit of this trial will be 'breast', and the trial will be powered towards 'number of breasts', so that the final number of included patients will depend on the proportion of patients undergoing bilateral breast reconstruction. The incidence of the primary endpoint is reported at 10%. With an α-level of 0.05, the trial will have a power of 0.90 to detect an absolute risk reduction of 5% if 1158 'breasts' are included. We will include patients, until a total number of 1274 breasts have completed the follow-up period of 180 days to account for a dropout rate of 10%. We estimate that 1003 patients will be included in the trial if approximately 27% of the patients undergo bilateral breast reconstruction.

8.2. Statistical analysis plan

The statistical analyses will be conducted on a modified intention-to-treat population, defined as all randomized patients with a valid informed consent. Categorical variables will be presented as frequencies whereas normally distributed continuous variables will be presented as mean ± SD, and as median (25^{th} percentile-75^{th} percentile) if non-normally distributed. Differences in endpoints (including the primary endpoint) between the treatment allocations will be analyzed with mixed effects models taking into account the correlation between breasts in patients undergoing bilateral surgery. The stratified randomization approach will ensure an even distribution of known endpoint risk factors between the placebo group and the treatment group. Logistic regression models will be applied to compare the odds of the primary endpoint between the two treatment groups. The models will be adjusted for potential confounders, including age, smoking, body mass index, indication for surgery (prophylactic mastectomy versus mastectomy after cancer diagnosis), immediate reconstruction versus delayed reconstruction, bilateral versus unilateral surgery, and radiotherapy status. In case of missingness greater than 5% for the primary outcome, we will apply multiple imputations by chained equations and repeat the primary analysis as sensitivity analysis. A statistical significance level of 0.05 will be applied throughout. The open source statistical program "R" (http://www.r-project.org) will be used for data treatment and statistical analysis.

8.3. Registration of changes

Changes to the original statistical analysis plan will be recorded in the sponsor's trial master file before unblinding.

9. Ethical considerations

This trial will be conducted in accordance with EU and national legislation on medical research in capable patient volunteers. Eligible subjects shall provide oral and written informed consent to participate in the trial. The informed consent can be withdrawn by the participant at any time during the trial after which the patient will convert to receiving treatment as determined by local guidelines. All data on trial participants will be protected according to the General Data Protection Regulation (GDPR), the data protection law and the Danish Health Act. The project is approved by the Danish Data Protection Agency and is to be approved by the Danish Medicines Agency and the Regional Committee on Health Research Ethics. The trial will be conducted according to national and international standards of good clinical practice (GCP) and will be monitored by the regional GCP unit.

9.1. Ethical justification

The trial will investigate the beneficial effects and potential side-effects of applying gentamicin, vancomycin and cefazolin locally onto the breast implant during breast reconstruction surgery. This may limit bacterial contamination of the implant and thereby decrease the risk of postoperative infection which is associated with a poor outcome for the patients. Therefore, participation in this trial could benefit the individual participant.

Inclusion in the trial may benefit the individual subject by decrease the risk of undergoing explantation of the implant, minimizing the risk of postoperative infection, minimize the hospitalization period and may as well improve the outcome for future women undergoing implant-based breast reconstructive surgery. Alternatively, we may find that the local antibiotics do not have a clinically relevant effect and perhaps negative side-effects that should be explored further.

The trial drug regimen is widely used internationally[14] but the potential positive and/or negative effects of using local antibiotics on breast implants have never been tested against placebo in a randomized trial. See appendix 1 for a review of the current literature.

Though the drug regimen has not been tested in a randomized controlled trial, the drugs have been applied by plastic surgeons for several years, and no adverse events has been reported.[42,44,48,56] Therefore, the drug regimen is expected to be of minimal risk to the subjects in the trial. Moreover, the systemic levels of antibiotics after locally applied antibiotics has been shown to be much lower than the levels seen with systemic antibiotics and the trial treatment consists of a single dose, and therefore we do not expect systemic adverse reactions to the trial drugs.[55]

Currently, no clinical guidelines exist in Denmark regarding the prophylactic use of local antibiotics to breast implants, and the treatment depends on the local approach at each hospital and the individual surgeon's preference. Allocation to placebo in this trial is therefore considered ethically acceptable. Application of local vancomycin on the breast implant has been used routinely at the Department of Plastic Surgery, Herlev and Gentofte Hospital, in the past years. This treatment regimen is not based on any evidence and is scientifically unjustified. Therefore, we find it ethically acceptable to include patients from Herlev and Gentofte Hospital in the trial. All eligible patients

from Herlev and Gentofte Hospital will receive this information before providing consent and specific written patient information material will be provided for these patients. Patients at Herlev and Gentofte Hospital who are not included in the trial will receive local vancomycin during the breast reconstruction surgery.

The results from this trial could change the guidelines for breast reconstruction surgery on an international level and be used to provide patients with evidence-based treatment.

9.2. Informed consent

Registered patients will receive information about the trial in their e-boks. They will be informed that the trial coordinating unit will contact them to provide oral information about the project after they have had time to read the information material about the trial. The contact information for registered patients will be passed on to the trial coordination unit from the treatment site.

Recruitment of trial participants will be carried out at the time of the preoperative patient visit or by telemedicine (i.e. telephone or a secure video connection) by a medical doctor with relevant expertise. All patients are encouraged to bring a third party (i.e. relative or partner) to this appointment. The responsible medical doctor can assign a designated nurse or medical student with relevant expertise to provide the patient with both oral and written information concerning the trial during this visit as the medical doctor has limited time available at the initial pre-operative consultation. The investigator carrying out the recruitment will be responsible for obtaining the informed consent (either by a digital consent or a written consent form) from the patients prior to any protocol-related activities. The conversation will take place in a private room behind a closed door. Participation in the trial will not influence the choice of treatment. The patient and the responsible medical doctor must personally provide a dated signature digitally or on a consent form. The informed consent can be withdrawn by the patient at any time and without explanation, after which the patient will receive the standard treatment according to the local guidelines. No personal data will be collected patients before informed consent is obtained.

9.2.1. General considerations

We will strive to provide the patients with at least 24 hours of consideration, but in some patients this will not be possible. For instance, when it comes to patients undergoing primary breast reconstruction, the breast reconstruction is performed during the same surgery as the cancer surgery and the patients are treated in an accelerated cancer treatment course. Due the short period of time from planning to carrying out the surgery, the decisions regarding possible use of implants or expander for reconstruction are often made a few hours before the surgery. In these cases, the patients will have at least 2 hours of consideration.

10. Direct access to source data/documentation

The site investigator will permit direct access to source data blinded for treatment allocation for monitoring, audits and reviews by the Health Ethics Committee, Good Clinical Practice unit, Danish Medicines Agency and other regulatory authorities.

11. Data management

All data management will be conducted according to guidelines of the Danish Data Protection Agency. The data will be kept in REDCap. The database will be maintained for 25 years from the last patient visit and anonymized when the approval from the Danish Data Protection Agency terminates.

12. Quality control and quality assurance

The trial will be conducted according to the approved protocol and will comply to standard procedures for quality control and quality assurance. The investigator at each trial site will report any deviations from standard protocol to the coordinating sponsor-investigator either by direct contact or via the case report form.

The trial conduct, data generation, data documentation and reporting will be in accordance with ICH-GCP guidelines and the trial will be monitored by the national Good Clinical Practice (GCP) unit. Monitoring will be conducted upon initiation of the trial, during the trial and at termination of the trial.

The sponsor-investigator, local trial site investigators and the trial coordinating unit are responsible for maintaining up-to-date accrual information, enrollment status and safety data. It is the responsibility of the sponsor-investigator to ensure oversight of trial related activities and on a yearly basis, report trial progression, enrollment status and safety data to the Trial Steering Committee (see Trial Steering Committee Charter). The sponsor-investigator will submit all relevant documents to the board members for scientific progress review. The sponsor-investigator is responsible for ensuring that access to clinical trial data is consistent with data protection principles and in accordance with the patients' informed consent provided in relation to their participation in the clinical trial.

The trial steering committee will be responsible for data monitoring and quality control of the data extracted from the patients' medical journals at the long-term follow-up after 5, 10 and 15 years.

13. Finance and insurance

The trial is funded by a project grant in surgical research of 2,975,000 DKK from the Novo Nordisk Foundation. The grant budget will cover a PhD salary, a part-time post-doc salary, and medicine and materials required for the trial. See attached research budget. The grant is disbursed to Professor Tine Engberg Damsgaard on behalf of Rigshospitalet and administered by the Financial Department of the Centre of Head and Orthopedics, Rigshospitalet. None of the researchers have any financial disclosures or relation to the Novo Nordisk Foundation. The Novo Nordisk Foundation have had no part in the design of the study, and they will not participate in the reporting of the results. The study participants will not be reimbursed for participation in the study. The trial participants are insured according to the Danish patient compensation scheme.

14. Publication plan

The trial is registered on EudraCT. The trial protocol will be registered at ClinicalTrials.gov before enrollment of the first patient. The protocol will be published prior to unblinding of the results in a methodology article including the statistical analysis plan. The main article will include the primary and secondary outcomes. The tertiary and long-term outcomes will be included in subsequent articles. The manuscripts will be used to report the results of the trial to the scientific community and will adhere to the CONSORT guidelines.[57] The members of the trial coordinating unit will be co-first authors and the coordinating sponsor-investigator will be senior/last author and corresponding author. All site investigators and members of the Trial Steering Committee will be co-authors. Other co-authorships will be decided by contribution according to the ICMJE authorship guidelines[58] depending on personal involvement. All publications will refer to the trial group which will include all contributing parties to the BREAST-AB trial. The manuscripts will be submitted to peer-reviewed international journals and both positive, negative and inconclusive results will be published. The findings of the trial will be shared with participating sites and presented at national and international conferences. The results will be disseminated to the public but will not be shared directly with participating patients. See Gant chart in figure 1.

15. Tasks and Responsibilities

Trial Coordinating Unit: Protocol development, daily management in the trial period, contact to Good Clinical Practice monitoring unit, contact to trial sites, data dictionary development, responsible for providing randomization sequences for each trial site, provide deidentified data to the trial steering committee for safety monitoring, available for unblinding the treatment allocation of individual patients in the case of an emergency, instructing health care personnel involved in the trial, data collection and management.

Coordinating Sponsor-Investigator: Overall responsibility for protocol development, funding, budget overview, ethical approval, trial registration, trial oversight (including enrollment trends and safety), contact to Good Clinical Practice monitoring unit, potential recruitment of additional sites, and dissemination and presentation of results.

Trial Steering Committee: Clinical and scientific advising to the sponsor-investigator, evaluation and recommendations based on yearly reports regarding enrollment trends, progress and safety of the trial, counseling regarding scientific reporting of data, data quality control during long-term follow-up. See 'Trial Steering Committee Charter' for a more thorough description of the tasks and responsibilities of the committee.

Site investigators: Responsible for site-specific enrollment, evaluation and reporting of eligible patients not included, education of personnel at trial sites, reporting of site-specific issues or challenges to the coordinating sponsor-investigator, inclusion of patients, responsible for obtaining informed consent, contact to the regional Good Clinical Practice monitoring unit, trial related data entry in the case report form

Clinical personnel: Preparation and administration of the trial drugs.

Good Clinical Practice-unit: See section 12.

Data Assessment Committee: Assessment and analysis of data.

16. Contact information

Contact for public queries

Mathilde Nejrup Hemmingsen, MD

Department of Plastic Surgery and Burns Treatment

Email: mathilde.nejrup.hemmingsen@regionh.dk

Phone: + 45 35 45 83 69

Contact for scientific queries

Mikkel Herly, MD

Department of Plastic Surgery and Burn Treatments

Blegdamsvej 9, DK2100 Copenhagen

E-mail: mikkel.herly@regionh.dk

Phone: +45 35 45 33 53

17. References

1. Bekæmpelse K. Statestik om brystkræft. Published 2018. https://www.cancer.dk/brystkraeft-mammacancer/statistik-brystkraeft/
2. 2018 Plastic Surgery Statistics. Published 2018. Accessed March 2, 2020. https://www.plasticsurgery.org/documents/News/Statistics/2018/plastic-surgery-statistics-full-report-2018.pdf
3. Coroneos CJ, Selber JC, Offodile AC, Butler CE, Clemens MW. US FDA breast implant postapproval studies: long-term outcomes in 99,993 patients. *Ann Surg*. 2019;269(1):30-36.
4. Stevens WG, Calobrace MB, Harrington J, Alizadeh K, Zeidler KR, d'Incelli RC. Nine-year core study data for Sientra's FDA-approved round and shaped implants with high-strength cohesive silicone gel. *Aesthetic Surg J*. 2016;36(4):404-416.
5. Maxwell GP, Van Natta BW, Bengtson BP, Murphy DK. Ten-year results from the Natrelle 410 anatomical form-stable silicone breast implant core study. *Aesthetic Surg J*. 2015;35(2):145-155.
6. Petersen A, Eftekhari ALB, Damsgaard TE. Immediate breast reconstruction: a retrospective study with emphasis on complications and risk factors. *J Plast Surg Hand Surg*. 2012;46(5):344-348.
7. Dikmans REG, Negenborn VL, Bouman M-B, et al. Two-stage implant-based breast reconstruction compared with immediate one-stage implant-based breast reconstruction augmented with an acellular dermal matrix: an open-label, phase 4, multicentre, randomised, controlled trial. *Lancet Oncol*. 2017;18(2):251-258.
8. Chopra K, Gowda AU, McNichols CHL, Brown EN, Slezak S, Rasko Y. Antimicrobial Prophylaxis Practice Patterns in Breast Augmentation. *Ann Plast Surg*. 2017;78(6):629-632. doi:10.1097/sap.0000000000000942
9. Yalanis GC, Liu E-W, Cheng H-T. Efficacy and safety of povidone-iodine irrigation in reducing the risk of capsular contracture in aesthetic breast augmentation: a systematic review and meta-analysis. *Plast Reconstr Surg*. 2015;136(4):687-698.
10. Kenna DM, Irojah BB, Mudge KL, Eveler K. Absorbable antibiotic beads prophylaxis in immediate breast reconstruction. *Plast Reconstr Surg*. 2018;141(4):486e-492e. doi:10.1097/PRS.0000000000004203
11. Nava MB, Catanuto G, Rocco N. Role of Mitomycin C in Preventing Capsular Contracture in Implant-Based Reconstructive Breast Surgery: A Randomized Controlled Trial. *Plast Reconstr Surg*. 2017;140(6):823e-824e. doi:10.1097/PRS.0000000000003851
12. Hunsicker LM, Chavez-Abraham V, Berry C, McEwen D. Efficacy of vancomycin-based continuous triple antibiotic irrigation in immediate, implant-based breast reconstruction. *Plast Reconstr Surg - Glob Open*. 2017;5(12). doi:10.1097/GOX.0000000000001624
13. Tutela JP, Duncan DP, Kelishadi SS, Chowdhry S, Boyd T, Little JA. Continuous Postoperative Antibiotic Irrigation via Catheter System Following Immediate Breast Reconstruction.

Eplasty. 2015;15:e49.

14. Hidalgo DA, Sinno S. Current trends and controversies in breast augmentation. *Plast Reconstr Surg*. 2016;137(4):1142-1150.

15. Lynch JM, Sebai ME, Rodriguez-Unda NA, Seal S, Rosson GD, Manahan MA. Breast pocket irrigation with antibiotic solution at implant insertion: a systematic review and meta-analysis. *Aesthetic Plast Surg*. 2018;42(5):1179-1186.

16. Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med*. 2013;158(3):200-207.

17. Pittet B, Montandon D, Pittet D. Infection in breast implants. *Lancet Infect Dis*. 2005;5(2):94-106. doi:10.1016/S1473-3099(05)01281-8

18. Bartsich S, Ascherman JA, Whittier S, Yao CA, Rohde C. The breast: a clean-contaminated surgical site. *Aesthetic Surg J*. 2011;31(7):802-806. doi:10.1177/1090820X11417428

19. Ahn CY, Ko CY, Wagar EA, Wong RS, Shaw WW. Microbial evaluation: 139 implants removed from symptomatic patients. *Plast Reconstr Surg*. 1996;98(7):1225-1229. doi:10.1097/00006534-199612000-00016

20. Pajkos A, Deva AK, Vickery K, Cope C, Chang L, Cossart YE. Detection of subclinical infection in significant breast implant capsules. *Plast Reconstr Surg*. 2003;111(5):1605-1611.

21. Rieger UM, Mesina J, Kalbermatten DF, et al. Bacterial biofilms and capsular contracture in patients with breast implants. *Br J Surg*. 2013;100(6):768-774.

22. Benediktsson K, Perbeck L. Capsular contracture around saline-filled and textured subcutaneously-placed implants in irradiated and non-irradiated breast cancer patients: Five years of monitoring of a prospective trial. *J Plast Reconstr Aesthetic Surg*. 2006;59(1):27-34. doi:10.1016/j.bjps.2005.08.005

23. Calobrace MB, Stevens WG, Capizzi PJ, Cohen R, Godinez T, Beckstrand M. Risk factor analysis for capsular contracture: A 10-year sientra study using round, smooth, and textured implants for breast augmentation. *Plast Reconstr Surg*. 2018;141(4S):20S-28S. doi:10.1097/PRS.0000000000004351

24. White IV RR, Pitzer KD, Fader RC, Rajab MH, Song J. Pharmacokinetics of topical and intravenous cefazolin in patients with clean surgical wounds. *Plast Reconstr Surg*. 2008;122(6):1773-1779.

25. Berberich C, Sanz-Ruiz P. Risk assessment of antibiotic resistance development by antibiotic-loaded bone cements: Is it a clinical concern? *EFORT Open Rev*. 2019;4(10):576-584. doi:10.1302/2058-5241.4.180104

26. Sweet FA, Roh M, Sliva C. Intrawound application of vancomycin for prophylaxis in instrumented thoracolumbar fusions: efficacy, drug levels, and patient outcomes. *Spine (Phila Pa 1976)*. 2011;36(24):2084-2088.

27. Adams Jr WP, Conner WCH, Barton Jr FE, Rohrich RJ. Optimizing breast-pocket irrigation: the post-betadine era. *Plast Reconstr Surg*. 2001;107(6):1596-1601.

28. Epps MT, Langsdon S, Pels TK, et al. Pocket Irrigation and Technique During Reconstructive Surgery: An American Society of Plastic Surgery Survey of Current Practice. *Ann Plast Surg*. 2019;82(6S Suppl 5):S427-S432. doi:10.1097/SAP.0000000000001790

29. Adams WP, Conner WCH, Barton FE, Rohrich RJ. Optimizing breast pocket irrigation: an in vitro study and clinical implications. *Plast Reconstr Surg*. 2000;105(1):334-338.

30. Darouiche RO, Meade R, Mansouri MD, Netscher DT. In vivo efficacy of antimicrobe-impregnated saline-filled silicone implants. *Plast Reconstr Surg*. 2002;109(4):1352-1357. doi:10.1097/00006534-200204010-00022

31. Zebala LP, Chuntarapas T, Kelly MP, Talcott M, Greco S, Riew KD. Intrawound vancomycin powder eradicates surgical wound contamination: an in vivo rabbit study. *J Bone Joint Surg Am*. 2014;96(1):46-51. doi:10.2106/JBJS.L.01257

32. Yarboro SR, Baum EJ, Dahners LE. Locally administered antibiotics for prophylaxis against surgical wound infection. An in vivo study. *J Bone Joint Surg Am*. 2007;89(5):929-933. doi:10.2106/JBJS.F.00919

33. Cavanaugh DL, Berry J, Yarboro SR, Dahners LE. Better prophylaxis against surgical site infection with local as well as systemic antibiotics. An in vivo study. *J Bone Joint Surg Am*. 2009;91(8):1907-1912. doi:10.2106/JBJS.G.01237

34. Frois AO, Harbour PO, Azimi F, et al. The role of antibiotics in breast pocket irrigation and implant immersion: a systematic review. *Plast Reconstr Surg Glob Open*. 2018;6(9).

35. Drinane JJ, Chowdhry T, Pham T-H, et al. Examining the Role of Antimicrobial Irrigation and Capsular Contracture: A Systematic Review and Meta-analysis. *Ann Plast Surg*. 2017;79(1):107-114. doi:http://dx.doi.org/10.1097/SAP.0000000000001134

36. Campbell CA. The Role of Triple-Antibiotic Saline Irrigation in Breast Implant Surgery. *Ann Plast Surg*. 2018;80(6S Suppl 6):S398-S402. doi:10.1097/SAP.0000000000001345

37. Horsnell JD, Searle AE, Harris PA, et al. Intra-operative techniques to reduce the risk of capsular contracture in patients undergoing aesthetic breast augmentation - A review. *Surgeon*. 2017;15(5):282-289. doi:http://dx.doi.org/10.1016/j.surge.2017.03.001

38. Huang N, Liu M, Yu P, Wu J. Antibiotic prophylaxis in prosthesis-based mammoplasty: a systematic review. *Int J Surg*. 2015;15:31-37.

39. Samargandi OA, Joukhadar N, Al Youha S, Thoma A, Williams J. Antibiotic Irrigation of Pocket for Implant-Based Breast Augmentation to Prevent Capsular Contracture: A Systematic Review. *Plast Surg (Oakville, Ont)*. 2018;26(2):110-119. doi:10.1177/2292550317747854

40. Burkhardt BR, Dempsey PD, Schnur PL, Tofield JJ. Capsular contracture: a prospective study of the effect of local antibacterial agents. *Plast Reconstr Surg*. 1986;77(6):919-932. https://www.ncbi.nlm.nih.gov/pubmed/3520619

41. Stevens WG, Nahabedian MY, Calobrace MB, et al. Risk factor analysis for capsular contracture: a 5-year Sientra study analysis using round, smooth, and textured implants for

breast augmentation. *Plast Reconstr Surg*. 2013;132(5):1115-1123.

42. Araco A, Gravante G, Araco F, Delogu D, Cervelli V, Walgenbach K. Infections of breast implants in aesthetic breast augmentations: a single-center review of 3,002 patients. *Aesthetic Plast Surg*. 2007;31(4):325-329.

43. Pfeiffer P, Jørgensen S, Kristiansen TB, Jørgensen A, Hölmich LR. Protective effect of topical antibiotics in breast augmentation. *Plast Reconstr Surg*. 2009;124(2):629-634.

44. Giordano S, Peltoniemi H, Lilius P, Salmi A. Povidone-iodine combined with antibiotic topical irrigation to reduce capsular contracture in cosmetic breast augmentation: a comparative study. *Aesthetic Surg J*. 2013;33(5):675-680.

45. Blount AL, Martin MD, Lineberry KD, Kettaneh N, Alfonso DR. Capsular contracture rate in a low-risk population after primary augmentation mammaplasty. *Aesthetic Surg J*. 2013;33(4):516-521.

46. Drinane JJ, Bergman RS, Folkers BL, Kortes MJ. Revisiting triple antibiotic irrigation of breast implant pockets: A placebo-controlled single practice cohort study. *Plast Reconstr Surg - Glob Open*. 2013;1(7). doi:10.1097/GOX.0b013e3182aa8760

47. Drinane JJ, Kortes MJ, Bergman RS, Folkers BL. Evaluation of antibiotic irrigation versus saline irrigation in reducing the long-term incidence and severity of capsular contraction after primary augmentation mammoplasty. *Ann Plast Surg*. 2016;77(1):32-36.

48. Adams Jr WP, Rios JL, Smith SJ. Enhancing patient outcomes in aesthetic and reconstructive breast surgery using triple antibiotic breast irrigation: six-year prospective clinical study. *Plast Reconstr Surg*. 2006;117(1):30-36.

49. Gutowski KA, Mesna GT, Cunningham BL. Saline-filled breast implants: A Plastic Surgery Educational Foundation multicenter outcomes study. *Plast Reconstr Surg*. 1997;100(4):1019-1027. doi:10.1097/00006534-199709001-00028

50. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC Definitions of Nosocomial Surgical Site Infections, 1992: A Modification of CDC Definitions of Surgical Wound Infections. *Infect Control Hosp Epidemiol*. 1992;13(10):606-608. doi:10.1086/646436

51. Spear SL, Baker JJL. Classification of capsular contracture after prosthetic breast reconstruction. *Plast Reconstr Surg*. 1995;96(5):1119-1123; discussion 1124.

52. Pusic AL, Klassen AF, Scott AM, Klok JA, Cordeiro PG, Cano SJ. Development of a new patient-reported outcome measure for breast surgery: the BREAST-Q. *Plast Reconstr Surg*. 2009;124(2):345-353.

53. Landspatientsregisteret: Avanceret udtræk. Published 2020. Accessed March 2, 2020. https://www.esundhed.dk/home/emner/operationer og diagnoser/landspatientsregisteret brugerdefineret udtraek#tabpanelFE2A577AEAAA41EE88D48460C9B1A8DD

54. Zalavras CG, Patzakis MJ, Holtom P. Local antibiotic therapy in the treatment of open fractures and osteomyelitis. *Clin Orthop Relat Res*. 2004;427:86-93.

55. Campbell CA. The Role of Triple-Antibiotic Saline Irrigation in Breast Implant Surgery. *Ann*

Plast Surg. 2018;80(6S Suppl 6):S398-S402. doi:10.1097/SAP.0000000000001345

56. Samargandi OA, Joukhdar N, Shaikh OM, Al Youha S, Williams J. Role of Antibiotic Irrigation in Preventing Capsular Contracture and Other Complication After Breast Augmentation: A Systematic Review and Meta-analysis. *Plast Reconstr Surg Glob Open*. 2016;4(9 Suppl).

57. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. *Int J Surg*. 2011;9(8):672-677. doi:10.1016/j.ijsu.2011.09.004

58. International Committee of Medical Journal Editors (ICMJE). International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals: Sample References. 2009;88(May):1-19.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	2
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
	2b	All items from the World Health Organization Trial Registration Data Set	4-18
Protocol version	3	Date and version identifier	Full protocol in supplementary material (SM)
Funding	4	Sources and types of financial, material, and other support	18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	3, 18
	5b	Name and contact information for the trial sponsor	3, 18
	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	18
	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)	18
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	6

	6b	Explanation for choice of comparators	Full protocol in SM
Objectives	7	Specific objectives or hypotheses	6, 9-10
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, noninferiority, exploratory)	7-8, 10-11
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7, full protocol in SM
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)	7-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	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)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7-8
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	9-11
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)	See Figure 2
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	11

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	NA
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	8-9
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	Full protocol in SM
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Full protocol in SM
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	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. Reference to where data collection forms can be found, if not in the protocol	9-11, full protocol in SM
	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	Full protocol in SM
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	Full protocol in SM

Statistical methods	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	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	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)	Full protocol in SM
Methods: Monitoring			
Data monitoring	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	11, Full protocol in SM
	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	Full protocol in SM
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12, Full protocol in SM
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Full protocol in SM
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4, 12
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)	4, full protocol in SM
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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	Full protocol in SM

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
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	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	Full protocol in SM
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
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	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: Protocol for a randomized, double-blind, placebo-controlled trial (The BREAST-AB trial)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-058697.R1
Article Type:	Protocol
Date Submitted by the Author:	04-Jul-2022

Complete List of Authors:	Hemmingsen, Mathilde; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Larsen, Andreas; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Weltz, Tim; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Ørholt, Mathias; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Wiberg, Sebastian; Zealand University Hospital Koge, Department of Anaesthesiology Bennedsen, Anne; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Bille, Camilla; Odense University Hospital, Department of Plastic Reconstructive Surgery Carstensen, Lena; Southwest Jutland Hospital, Department of Plastic Surgery Jensen, Lisa; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Bredgaard, Rikke; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Herlev og Gentofte Koudahl, Vibeke; Lillebaelt Hospital, Department of Plastic Surgery Schmidt, Volker ; Zealand University Hospital Roskilde, Department of Plastic and Breast Surgery; University of Copenhagen, Department of Clinical Medicine Vester-Glowinski, Peter; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Hölmich, Lisbet; Copenhagen University Hospital, Department of Plastic and Reconstructive Surgery, Herlev og Gentofte; University of Copenhagen, Department of Clinical Medicine Sørensen, Søren; University of Copenhagen, Department of Biology, Section of Microbiology Bjarnsholt, Thomas; University of Copenhagen, Department of Immunology and Microbiology; Copenhagen University Hospital, Department of Clinical Microbiology, Rigshospitalet Damsgaard, Tine; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet; University of Copenhagen, Department of Clinical Medicine

	Herly, Mikkel; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet; University of Copenhagen, Department of Immunology and Microbiology
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Evidence based practice
Keywords:	Plastic & reconstructive surgery < SURGERY, Breast surgery < SURGERY, Clinical trials < THERAPEUTICS

Licence statement

***** I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in BMJ Open and any other BMJ products and to exploit all rights, as set out in our licence.

☑

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Title: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: Protocol for a randomized, double-blind, placebo-controlled trial (The BREAST-AB trial)

Authors

Mathilde Nejrup Hemmingsen, MD[1]; Andreas Larsen, MD[1]; Tim Kongsmark Weltz, MD[1]; Mathias Ørholt Nielsen, MD[1]; Sebastian Wiberg, MD[2]; Anne Karen Bennedsen, BMSc[1]; Camilla Bille, MD[3]; Lena Felicia Carstensen, MD[4]; Lisa Toft Jensen, MD[1]; Rikke Bredgaard, MD[5]; Vibeke Koudahl, MD[6]; Volker Jürgen Schmidt, MD[7,8]; Peter Viktor Vester-Glowinski, MD[1]; Lisbet Rosenkrantz Hölmich[5,8], MD; Søren Johannes Sørensen, MSc[9]; Thomas Bjarnsholt, MSc[10,11]; Tine Engberg Damsgaard, MD[1,8]; Mikkel Herly, MD[1,10]

Affiliations

[1]Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet, Copenhagen, Denmark

[2] Zealand University Hospital Køge, Department of Anaesthesiology, Køge, Denmark

[3]Odense University Hospital, Department of Plastic Reconstructive Surgery, Odense, Denmark

[4]Southwest Jutland Hospital, Department of Plastic Surgery, Esbjerg, Denmark

[5]Copenhagen University Hospital, Department of Plastic and Reconstructive Surgery, Herlev and Gentofte, Herlev, Denmark

[6]Lillebaelt Hospital, Department of Plastic Surgery, Vejle, Denmark

[7]Zealand University Hospital Roskilde, Department of Plastic and Breast Surgery, Roskilde, Denmark

[8]University of Copenhagen, Department of Clinical Medicine, Copenhagen, Denmark

[9]University of Copenhagen, Department of Biology, Section of Microbiology, Copenhagen, Denmark

[10]University of Copenhagen, Department of Immunology and Microbiology, Copenhagen, Denmark

[11]Copenhagen University Hospital, Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

Corresponding author

Mathilde Nejrup Hemmingsen, MD

Department of Plastic Surgery and Burns Treatment

Copenhagen University Hospital, Rigshospitalet

Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Email: mathilde.nejrup.hemmingsen@regionh.dk

Phone: +45 285 682 05

Keywords: Breast reconstruction, implant irrigation, infection, capsular contracture

Word count: 2.899

Abstract (257/300)

Introduction

Periprosthetic infection is one of the most severe complications following implant-based breast reconstruction affecting 5-10% of the women. Currently, many surgeons apply antibiotics locally on the breast implant to reduce the risk of postoperative infection, but no randomized, placebo-controlled trials have tested the treatment's efficacy.

Methods and analysis

The BREAST-AB trial is an investigator-initiated, multicentre, randomized, placebo-controlled, double-blind trial of local treatment with gentamicin, vancomycin and cefazolin on breast implants in women undergoing implant-based breast reconstruction. The trial drug consists of 80 mg gentamicin, 1 g vancomycin and 1 g cefazolin dissolved in 500 ml of isotonic saline. The placebo solution consists of 500 ml isotonic saline. The trial drug is used to wash the dissected tissue pocket and the breast implant prior to insertion. The primary outcome is all-cause explantation of the breast implant within 180-days after the breast reconstruction surgery. This excludes cases where the implant is replaced with a new permanent implant e.g., for cosmetic reasons. Key long-term outcomes include capsular contracture and quality of life. The trial started on 26 January 2021 and is currently recruiting.

Ethics and dissemination

The trial was approved by the Regional Ethics Committee of the Capital Region (H-20056592) on 1 January 2021 and the Danish Medicines Agency (2020070016) on 2 August 2020. The main paper will include the primary and secondary outcomes and will be submitted to an international peer-reviewed journal.

Registration

The trial was registered at ClinicalTrials.gov (NCT 04731025) on 29 January 2021 and at the EU Clinical Trials Register (EudraCT 2020-002459-40) on 17 December 2020.

Strengths and limitations of this study

- The trial will include all types of patients undergoing breast reconstruction surgery with implants which makes it relevant for all women undergoing implant-based breast reconstruction
- The women undergoing bilateral breast reconstruction will receive antibiotic treatment to one of their breasts and placebo to the contralateral breast which isolate the effect of the trial drug from inter-individual variation
- The trial is evaluated with endpoints that are of great importance for patients
- The incidence of the primary outcome is relatively low, so despite the large sample size, a small effect of the treatment may not be detected with statistical significance

Text (2.899/4.000 words)

Introduction

Breast reconstruction has been shown to improve a women's quality of life after undergoing breast cancer surgery.[1] An increasing number of women choose implant-based breast reconstruction[2] which includes a risk of implant infection. Implant infection is seen in 5-10% of the women,[3]–[7] and the treatment typically requires removal of the implant after which the patient must wait several months before a new breast reconstruction can be attempted.

Previous studies suggest that bacterial contamination of the breast implant can occur without any clinical symptoms.[8], [9] Instead, the bacteria form a chronic, subclinical infection which is suspected to cause a prolonged immune reaction to the implant called capsular contracture which affects up to 10-20% of the patients.[10], [11] Capsular contracture causes hardening and deformity of the breast, and the treatment often includes surgical removal of the contracted capsule and exchange of the implant.

Surgeons have attempted numerous strategies to prevent bacterial contamination of the implant.[12]–[17] The most widely followed approach is to apply antibiotics directly on the breast implant and in the dissected tissue pocket during the surgery,[18] but only few studies have investigated the clinical effect of the treatment. A recent meta-analysis found a decreased rate of implant infection and capsular contracture in women treated with antibiotics applied on the breast implant.[19] However, the included studies were mostly retrospective and varied greatly in the applied antibiotics, control groups and follow-up period. Furthermore, no randomized controlled trials were identified in the meta-analysis.[19]

Due to the limited evidence, The Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection has no recommendations regarding the use of locally applied antibiotics on implants[20] and The National Institute for Health and Care Excellence (NICE) in England has requested further studies investigating the clinical effect of the treatment.[21] Randomized clinical trials are essential for developing evidence-based treatment guidelines. The BREAST-AB trial is designed to assess the effect of locally applied antibiotics on all-cause loss of the implant after implant-based breast reconstruction. We hypothesize that local application of gentamicin, vancomycin and cefazolin decrease the risk of postoperative clinical infections and

thereby reduce the risk of losing the implant to the benefit of women undergoing implant-based breast reconstruction.

Methods and analysis

The protocol was written in accordance with the SPIRIT statement[22] and the ICH-GCP guidelines[23]. The protocol is provided in full length in supplementary file 1

Trial design

The BREAST-AB trial is an investigator-initiated, multicentre, randomized, double-blind, placebo-controlled trial investigating local application of gentamicin, vancomycin and cefazolin during implant-based breast reconstruction. The antibiotic solution or placebo is applied directly onto the breast implant and in the dissected tissue pocket during the surgery.

Setting

The trial will be conducted at six hospitals in Denmark, and additional trial sites may be included during the trial period. See supplementary file 1 for a list of the trial sites.

Eligibility criteria

Patients that meet the following criteria are considered eligible for inclusion:

- Age ≥ 18
- Biologically female
- Written informed consent
- Scheduled for breast reconstruction with implants or expanders including
  - immediate or delayed reconstruction
  - unilateral or bilateral reconstruction
  - with or without simultaneous flap reconstruction

Exclusion criteria are:

- Pregnancy
- Breast feeding
- Known allergy towards gentamicin, vancomycin, cefazolin or neomycin

- Known anaphylactic reaction towards beta-lactam antibiotics or aminoglycosides
- Myasthenia gravis
- Known impaired renal function, GFR < 60 ml/min
- Participation in investigational drug trials concerning disinfection agents in the breast cavity

Trial intervention

The trial drug contains 80 mg gentamicin, 1000 mg vancomycin and 1000 mg cefazolin dissolved in an infusion bag containing 500 ml of sterile isotonic saline. The placebo solution consists of 500 ml sterile isotonic saline contained in a similar infusion bag. Both solutions are achromatic, and the infusion bags are indistinguishable from one another. See figure 1 for an illustration of the trial intervention.

During the surgery, the responsible nurse draws 150 ml from the assigned infusion bag and the plastic surgeon use it to wash the dissected tissue pocket.[24] Another 50 ml is drawn from the same infusion bag and used to soak the implant prior to insertion in the tissue pocket. The rest of the content in the infusion bag is discarded.

Randomization

The trial drug and placebo are assigned in a 1:1 ratio. Patients undergoing unilateral breast reconstruction are randomized to either the trial drug or placebo, whereas patients who undergo bilateral breast reconstruction are randomized to the trial drug on one breast and placebo on the contralateral breast. The paired design in the patients undergoing bilateral surgery isolates the effect of the trial treatment from the inter-individual variation, as these patients serve as their own control. Patients who undergo two-stage breast reconstruction with an expander implant that is replaced with a permanent implant after three to six months are allocated to the same trial treatment during both surgeries. See figure 2 for an overview of the trial design.

The randomization is stratified according to study site, whether the patients undergo unilateral- or bilateral surgery and selected risk factors based on the literature[25] including radiation therapy and immediate- versus delayed reconstruction. This approach ensures an even distribution of the selected risk factors in the placebo group and the intervention group. The randomized design will

ensure that other potential risk factors, which are not included in the stratification, are evenly distributed in the intervention- and control group. The treatment is assigned in a fixed block size of two to ensure that the trial drug and placebo is evenly distributed within each stratum.

Blinding

The trial is double-blind so that the patients, site investigators, health care personnel and the data assessors are blinded to the allocated treatment. The only unblind investigators are the nurses responsible for preparing the trial drugs and the members of the trial coordination unit who provide the treatment allocation. The designated nurse prepares the trial drugs before the surgery. The trial drugs are prepared outside of the operating room to make sure that the surgeon and the surgical staff are blinded to the treatment. The unblind investigators do not take part in any treatment-related procedures, clinical evaluation of the outcomes or data assessment. In case of emergency unblinding, the trial coordination unit will provide the allocation assignment under discretion of the treating physician.

Primary outcome

All-cause explantation of the breast implant within 180-days after the breast reconstruction

All-cause explantation is defined as explantation and discarding of the breast implant. However, the following cases are not counted as explantation: replacement of an expander with a permanent implant; and replacement of a permanent breast implant with a new permanent breast implant due to cosmetic revisions such as asymmetry, implant malposition, change of size or implant rotation.

The rationale for the primary outcome is to quantify whether the locally applied antibiotics prevent severe infection or other complications that leads to loss of the reconstruction within 180 days after surgery. Sometimes, the indication for explantation of the implant may be ambiguous because multiple complications can occur simultaneously. Therefore, all-cause explantation was chosen as a more objective alternative to infection that leads to explantation of the implant. The primary outcome does not include explantation and direct placement of a new permanent implant for cosmetic reasons because revisional surgery is not considered a proxy for severe complications that may be affected by antibiotics on the implant.

The definition of explantation for cosmetic reasons and discarding of the breast implant was revised in the protocol V2.8, dated 10 May 2022, from only mentioning asymmetry and implant rotation to include all types of implant malposition and change of implant size.

Secondary outcomes

Time to explantation

Time to explantation is defined as the number of days from the reconstructive surgery to the surgical removal of the implant. This outcome was chosen because local application of antibiotics may delay the development of a postoperative clinical infection.

All-cause explantation of the breast implant within 1 year after the breast reconstruction surgery (Y/N)

Previous studies[26] suggest that surgical removal of the permanent implant can occur up to 1 year after the surgery, and therefore this is included as a secondary outcome.

Revision surgery with incision of the fibrous capsule after the breast reconstruction surgery

This is defined as all revisional surgery that includes exposure of the breast implant. This outcome was included because the breast reconstruction in some cases can be upheld with revisional surgery despite complications that may be associated with low-virulent bacteria.

Exchange of the permanent implant with an expander implant after the breast reconstructive surgery

This subgroup consists of patients who undergo a salvage procedure, where the permanent implant is removed and discarded, and the implant pocket is cleansed and irrigated with antiseptic agents (e.g., a solution of hydrogen peroxide) after which an expander implant is inserted to prevent the tissue envelope from contracting while still preserving the vulnerable skin flaps. This group will be counted in the primary outcome, but we hypothesize that the patients chosen for this treatment option may have less severe symptoms of infection and may have concomitant necrosis due to poor blood supply to the skin flaps. Therefore, the effect of the treatment in this subgroup may be modest compared to the patients who have explantation without replacing it with an expander.

Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction

Surgical site infection is defined according to the CDC classification.[27] The clinical signs of infection is combined with the prescription of antibiotics as a confirmation of the surgeons suspicion of infection. Additional outcome measures are listed in supplementary file 1 and on ClinicalTrials.gov.

Long-term outcomes

The trial includes a long-term assessment of capsular contracture after 5, 10 and 15 years. The use of locally applied antibiotics could potentially decrease the rate of capsular contracture by minimizing or altering the low-virulent bacterial contamination of the implant. Therefore, capsular contracture is an important long-term outcome.

The trial also evaluates long-term quality of life using the BREAST-Q questionnaire 'Reconstruction Module'.[28] The preoperative questionnaire is administered after the patient has provided informed consent. The postoperative questionnaire is administered 3 months, 1 year and 5 years postoperatively. The application of local antibiotics may decrease the risk of postoperative complications and thereby decrease the risk of undergoing revision surgery. This in turn may lead to improved patient satisfaction and quality of life. BREAST-Q is a validated tool used to quantify patient satisfaction and health-related quality of life after breast reconstruction surgery.

Sample size

The trial is powered to find a 5% risk reduction in the primary outcome. Based on the literature, the assumed rate of implant loss in the control group is 10%.[6], [7] The independent sample unit is "breast", because previous data does not suggest that implant loss is correlated between the two breasts of a patient.[3] Therefore, the power of the trial is based on the number of breasts, so that the final number of included patients depends on the proportion of patients who undergo bilateral breast reconstruction. With an alpha of 0.05, the trial will have a power of 0.90 to detect an absolute risk reduction of 5% with 1158 breasts. To account for drop-out of up to 10%, we will include patients with a combined estimated number of 1274 breasts in the trial. We expect 27% of

the patients to undergo bilateral breast reconstruction (based on unpublished data) and therefore, 1003 patients will be included in the trial.

Statistical analysis plan

The statistical analyses and -reporting will adhere to the CONSORT guidelines.[29] All statistical analyses will be conducted on a modified intention-to-treat population defined as all patients that have been allocated to the study drug and have a valid informed consent.

The primary outcome and key secondary outcomes are categorical variables and will be presented as frequencies in each group. The overall effect of the intervention on the primary and secondary outcomes will be modelled as both univariate and multivariate mixed effects logistic regression models considering the correlation between breasts in patients undergoing bilateral surgery. The results will be presented as crude and adjusted odds ratios (OR) with 95% confidence intervals. The model will be adjusted for potential confounders, including age, smoking, body mass index, trial site and indication for surgery (prophylactic mastectomy versus mastectomy after cancer diagnosis). The full statistical analysis plan is provided in the protocol in supplementary file 1.

Data collection and follow-up

All patients are admitted to the hospital for approximately 3 days after the surgery. All patients are scheduled for postoperative follow-up visits after approximately 3 months and 1 year. Data on drug administration is obtained real-time and entered in an electronic case report form. Additional data is obtained from the patients' medical records by trained researchers and entered in the electronic case report form. A list of included variables is provided in the protocol in supplementary file 1.

Clinical treatment

Participation in the trial will not interfere with any clinical decisions regarding the treatment of the patients, and all other clinical treatment, including pre-, peri- and postoperatively administered medicine, will adhere to the standard treatment at each trial site.

Patient and public involvement

None.

Ethics and dissemination

Ethical considerations

The trial protocol has been reviewed and approved by the Regional Ethics Committee of the Capital Region (H-20056592) on 1 January 2021 and the Danish Medicines Agency (EudraCT 2020-002459-40) on 2 August 2020. The trial is monitored by the Good Clinical Practice units in Denmark.

There are currently no clinical guidelines in Denmark regarding the use of locally applied antibiotics on breast implants, and the treatment depends on the individual surgeon's preference. Allocation to placebo in this trial is therefore considered ethically acceptable. A detailed description of the ethical considerations is provided in the supplementary file 1.

Safety considerations

Previous studies have shown that the serum level after local application of antibiotics is low,[30], [31] and therefore the risk of systemic side effects is low. Gentamicin, vancomycin and cefazolin have been used for local application on breast implants for many years and are considered safe.[16], [32] If the patients experience adverse events, it will be registered in an electronic case report form in REDCap and treated according to local guidelines. All serious adverse reactions will be reported yearly to the Danish Medicines Agency and the Regional Ethics Committee by the sponsor-investigator during the study period. A more detailed description of the safety considerations is provided in the protocol in supplementary file 1.

Consent

Consent from trial participants is obtained according to Danish legislation.[33] The investigators are responsible for obtaining the signed, informed consent from the patients prior to any protocol-related activities. The consent can be withdrawn by the patient at any time and without explanation, after which the patient will receive the standard treatment according to the local guidelines. An example of the patient consent form is provided in supplementary file 2 and 3.

Dissemination

The main paper will include the primary and secondary outcomes. The manuscript will adhere to the CONSORT guidelines and will be used to report the results of the trial to the scientific community. The manuscript will be submitted to an international peer-reviewed journal, and both positive, negative and inconclusive results will be published. The findings of the trial will be shared with participating sites and presented at national and international conferences. The results will be registered at ClinicalTrials.gov and will be disseminated to the public.

Status

The first patient was enrolled in the trial in January 2021, and the trial is currently recruiting. The last patient is expected to be included in January 2025. The primary results of the trial are anticipated in July 2025 after the last patient's last follow-up. The results from this trial can be used in evidence-based treatment guidelines for implant-based breast reconstruction surgery.

Author contributions

All authors took part in designing the trial and writing the trial protocol. Mathilde Nejrup Hemmingsen, Andreas Larsen, Tim K Weltz and Anne K Bennedsen constitutes the trial coordinating unit who are responsible for coordinating all trial related activities. Mathilde Nejrup Hemmingsen has written the manuscript. Mikkel Herly is the Coordinating Principal Investigator of the trial and the local site investigator at Rigshospitalet. He has critically revised the manuscript and contributed to the final version of the manuscript. Tine Damsgaard, Søren J Sørensen, Thomas Bjarnsholt, Peter Vester-Glowinski and Mikkel Herly are members of the trial steering committee and they have all revised and approved the final manuscript. Camilla Bille, Rikke Bredgaard, Lena F Carstensen, Lisbet Rosenkrantz Hölmich, Lisa Toft Jensen, Vibeke Koudahl and Volker J Schmidt are local site investigators and they have revised and approved the final manuscript. Mathias Ørholt and Sebastian Wiberg are members of the blinded data assessment committee and have provided statistical expertise to the trial design and contributed with writing the statistics sections in the manuscript. All authors have read and approved the final manuscript.

Competing interests

None declared.

Funding and sponsor

Mikkel Herly is the initiator and sponsor of the trial. The trial is supported by the Novo Nordisk Foundation (grant number 0058322, grant holder Tine Damsgaard) and the Medicine Fund of the Danish Regions (grant number R-189-A4127, grant holder Mikkel Herly).

Data sharing statement

After publication of the results, researchers and other relevant parties can be granted access to anonymized data upon request.

References

[1] Y. Eltahir *et al.*, "Quality-of-life outcomes between mastectomy alone and breast reconstruction: comparison of patient-reported BREAST-Q and other health-related quality-of-life measures," *Plast. Reconstr. Surg.*, vol. 132, no. 2, pp. 201e-209e, 2013.

[2] American Society of Plastic Surgeons, "2019 Plastic Surgery Statistics Report," 2019. [Online]. Available: https://www.plasticsurgery.org/documents/News/Statistics/2019/plastic-surgery-statistics-full-report-2019.pdf.

[3] C. J. Coroneos, J. C. Selber, A. C. Offodile, C. E. Butler, and M. W. Clemens, "US FDA breast implant postapproval studies: long-term outcomes in 99,993 patients," *Ann. Surg.*, vol. 269, no. 1, pp. 30–36, 2019.

[4] W. G. Stevens, M. B. Calobrace, K. Alizadeh, K. R. Zeidler, J. L. Harrington, and R. C. d'Incelli, "Ten-year Core Study Data for Sientra's Food and Drug Administration–Approved Round and Shaped Breast Implants with Cohesive Silicone Gel," *Plast. Reconstr. Surg.*, vol. 141, no. 4S, pp. 7S-19S, 2018.

[5] G. P. Maxwell, B. W. Van Natta, B. P. Bengtson, and D. K. Murphy, "Ten-year results from the Natrelle 410 anatomical form-stable silicone breast implant core study," *Aesthetic Surg. J.*, vol. 35, no. 2, pp. 145–155, 2015.

[6] A. Petersen, A. L. B. Eftekhari, and T. E. Damsgaard, "Immediate breast reconstruction: a retrospective study with emphasis on complications and risk factors," *J. Plast. Surg. Hand Surg.*, vol. 46, no. 5, pp. 344–348, 2012.

[7] R. E. G. Dikmans *et al.*, "Two-stage implant-based breast reconstruction compared with immediate one-stage implant-based breast reconstruction augmented with an acellular dermal matrix: an open-label, phase 4, multicentre, randomised, controlled trial," *Lancet Oncol.*, vol. 18, no. 2, pp. 251–258, 2017.

[8] A. Pajkos, A. K. Deva, K. Vickery, C. Cope, L. Chang, and Y. E. Cossart, "Detection of subclinical infection in significant breast implant capsules," *Plast. Reconstr. Surg.*, vol. 111,

no. 5, pp. 1605–1611, 2003.

[9] U. M. Rieger *et al.*, "Bacterial biofilms and capsular contracture in patients with breast implants," *Br. J. Surg.*, vol. 100, no. 6, pp. 768–774, 2013.

[10] K. Benediktsson and L. Perbeck, "Capsular contracture around saline-filled and textured subcutaneously-placed implants in irradiated and non-irradiated breast cancer patients: Five years of monitoring of a prospective trial," *J. Plast. Reconstr. Aesthetic Surg.*, vol. 59, no. 1, pp. 27–34, 2006, doi: 10.1016/j.bjps.2005.08.005.

[11] M. B. Calobrace, W. G. Stevens, P. J. Capizzi, R. Cohen, T. Godinez, and M. Beckstrand, "Risk factor analysis for capsular contracture: A 10-year sientra study using round, smooth, and textured implants for breast augmentation," *Plast. Reconstr. Surg.*, vol. 141, no. 4S, pp. 20S-28S, 2018, doi: 10.1097/PRS.0000000000004351.

[12] K. Chopra, A. U. Gowda, C. H. L. McNichols, E. N. Brown, S. Slezak, and Y. Rasko, "Antimicrobial Prophylaxis Practice Patterns in Breast Augmentation: A National Survey of Current Practice.," *Ann. Plast. Surg.*, vol. 78, no. 6, pp. 629–632, Jun. 2017, doi: 10.1097/SAP.0000000000000942.

[13] G. C. Yalanis, E.-W. Liu, and H.-T. Cheng, "Efficacy and safety of povidone-iodine irrigation in reducing the risk of capsular contracture in aesthetic breast augmentation: a systematic review and meta-analysis," *Plast. Reconstr. Surg.*, vol. 136, no. 4, pp. 687–698, 2015.

[14] D. M. Kenna, B. B. Irojah, K. L. Mudge, and K. Eveler, "Absorbable antibiotic beads prophylaxis in immediate breast reconstruction," *Plast. Reconstr. Surg.*, vol. 141, no. 4, pp. 486e-492e, 2018, doi: 10.1097/PRS.0000000000004203.

[15] M. B. Nava, G. Catanuto, and N. Rocco, "Reply: Role of Mitomycin C in Preventing Capsular Contracture in Implant-Based Reconstructive Breast Surgery: A Randomized Controlled Trial," *Plast. Reconstr. Surg.*, vol. 140, no. 6, pp. 823e-824e, 2017, doi: 10.1097/PRS.0000000000003851.

[16] L. M. Hunsicker, V. Chavez-Abraham, C. Berry, and D. McEwen, "Efficacy of vancomycin-based continuous triple antibiotic irrigation in immediate, implant-based breast

reconstruction," *Plast. Reconstr. Surg. - Glob. Open*, vol. 5, no. 12, 2017, doi: 10.1097/GOX.0000000000001624.

[17] J. P. Tutela, D. P. Duncan, S. S. Kelishadi, S. Chowdhry, T. Boyd, and J. A. Little, "Continuous Postoperative Antibiotic Irrigation via Catheter System Following Immediate Breast Reconstruction.," *Eplasty*, vol. 15, p. e49, 2015.

[18] D. A. Hidalgo and S. Sinno, "Current trends and controversies in breast augmentation," *Plast. Reconstr. Surg.*, vol. 137, no. 4, pp. 1142–1150, 2016.

[19] J. M. Lynch, M. E. Sebai, N. A. Rodriguez-Unda, S. Seal, G. D. Rosson, and M. A. Manahan, "Breast pocket irrigation with antibiotic solution at implant insertion: a systematic review and meta-analysis," *Aesthetic Plast. Surg.*, vol. 42, no. 5, pp. 1179–1186, 2018.

[20] S. I. Berriós-Torres *et al.*, "Centers for disease control and prevention guideline for the prevention of surgical site infection, 2017," *JAMA Surg.*, vol. 152, no. 8, pp. 784–791, 2017, doi: 10.1001/jamasurg.2017.0904.

[21] National Institute for Health and Care Excellence (NICE), "Surgical site infections: prevention and treatment." https://www.nice.org.uk/guidance/ng125/chapter/Recommendations#intraoperative-phase.

[22] A.-W. Chan *et al.*, "SPIRIT 2013 statement: defining standard protocol items for clinical trials," *Ann. Intern. Med.*, vol. 158, no. 3, pp. 200–207, 2013.

[23] I. C. H. H. T. Guideline, "General considerations for clinical trials E8," in *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use*, 1997.

[24] W. P. Adams Jr, J. L. Rios, and S. J. Smith, "Enhancing patient outcomes in aesthetic and reconstructive breast surgery using triple antibiotic breast irrigation: six-year prospective clinical study," *Plast. Reconstr. Surg.*, vol. 117, no. 1, pp. 30–36, 2006.

[25] M. A. Olsen, K. B. Nickel, I. K. Fox, J. A. Margenthaler, A. E. Wallace, and V. J. Fraser, "Comparison of Wound Complications After Immediate, Delayed, and Secondary Breast

Reconstruction Procedures.," *JAMA Surg.*, vol. 152, no. 9, p. e172338, Sep. 2017, doi: 10.1001/jamasurg.2017.2338.

[26] J. Kalstrup, C. B. Willert, M. B.-M. Weitemeyer, A. H. Chakera, and L. R. Hölmich, "Immediate direct-to-implant breast reconstruction with acellular dermal matrix: Evaluation of complications and safety," *The Breast*, vol. 60, pp. 192–198, 2021.

[27] H. TC, "Gaynes RP. Martone WJ. Jarvis WR. Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections," *Infect Control Hosp Epidemiol*, vol. 13, pp. 606–608, 1992.

[28] A. L. Pusic, A. F. Klassen, A. M. Scott, J. A. Klok, P. G. Cordeiro, and S. J. Cano, "Development of a new patient-reported outcome measure for breast surgery: the BREAST-Q," *Plast. Reconstr. Surg.*, vol. 124, no. 2, pp. 345–353, 2009.

[29] K. F. Schulz, D. G. Altman, D. Moher, and C. Group, "CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials," *Trials*, vol. 11, no. 1, p. 32, 2010.

[30] F. A. Sweet, M. Roh, and C. Sliva, "Intrawound application of vancomycin for prophylaxis in instrumented thoracolumbar fusions: efficacy, drug levels, and patient outcomes," *Spine (Phila. Pa. 1976).*, vol. 36, no. 24, pp. 2084–2088, 2011.

[31] R. R. White IV, K. D. Pitzer, R. C. Fader, M. H. Rajab, and J. Song, "Pharmacokinetics of topical and intravenous cefazolin in patients with clean surgical wounds," *Plast. Reconstr. Surg.*, vol. 122, no. 6, pp. 1773–1779, 2008.

[32] C. A. Campbell, "The Role of Triple-Antibiotic Saline Irrigation in Breast Implant Surgery," *Ann. Plast. Surg.*, vol. 80, no. 6S Suppl 6, pp. S398–S402, Jun. 2018, doi: 10.1097/SAP.0000000000001345.

[33] Retsinformation, "Lov om et videnskabsetisk komitesystem og behandling af biomedicinske forskningsprojekter," *30/05/2003*, 2003. https://www.retsinformation.dk/eli/lta/2003/402 (accessed Apr. 19, 2021).

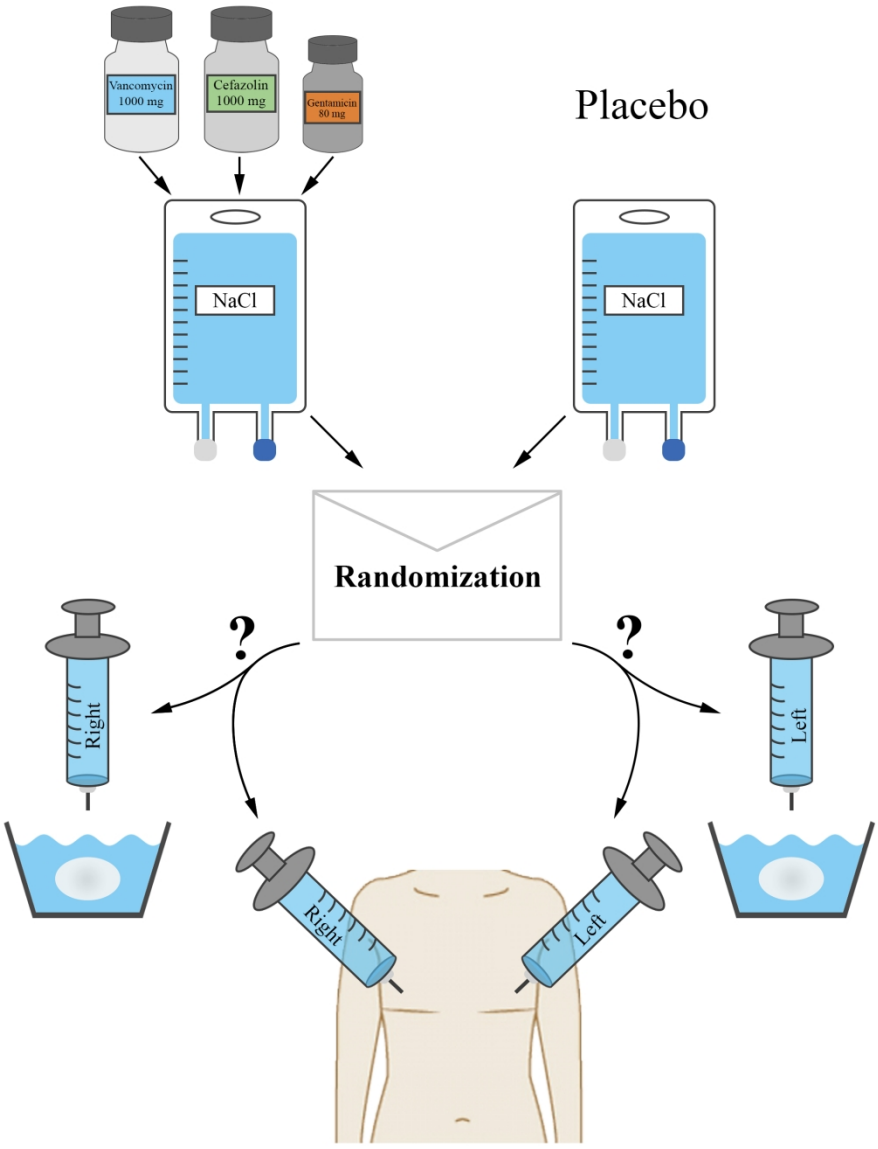


Figure 1: Illustration of the trial intervention. The trial drug contains 1000 mg Vancomycin, 1000 mg Cefazolin and 80 mg Gentamicin dissolved in an infusion bag containing 500 ml of sterile isotonic saline. The placebo solution consists of 500 ml sterile isotonic saline.

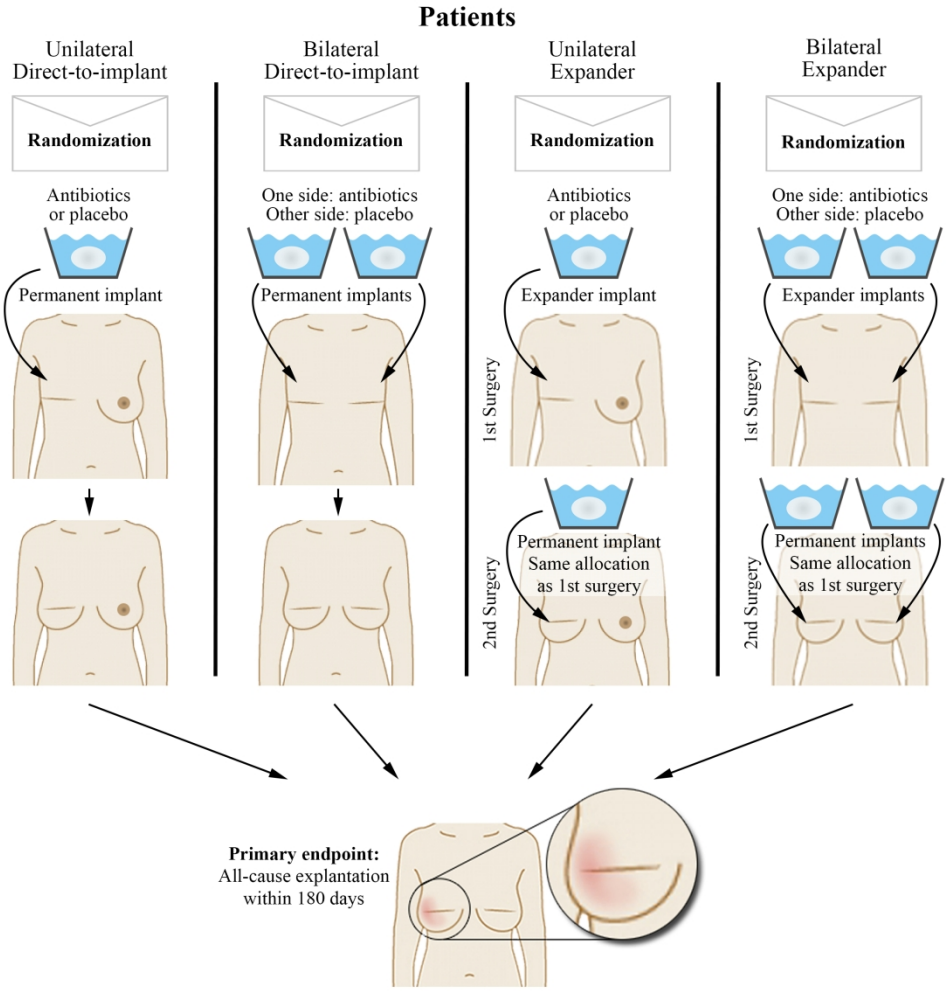


Figure 2. Overview of the trial design. The patients are randomized to antibiotic treatment or placebo applied directly onto the breast implant and in the dissected tissue pocket. Patients who undergo bilateral breast reconstruction are randomized to antibiotics on one side and placebo on the contralateral side. Patients who undergo unilateral breast reconstruction are randomized to either antibiotics or placebo.

Version 2.8
May 10, 2022

Trial Protocol

Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Short Title: Local Antibiotics for Breast Implants

Acronym: The Breast-AB Trial

By

Mikkel Herly, MD
Mathilde Hemmingsen, MD
Andreas Larsen, BMSc
Tim Weltz, BMSc

Version: 2.8 (10/5/22)

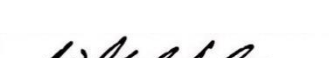
Sponsor Protocol Code Number: BREAST-AB-01

EudraCT number: 2020-002459-40

Date of Registration: 05-12-2020

Funding number: Novo Nordisk Foundation 0058322

Coordinating Sponsor-Investigator:
Mikkel Herly, MD
Department of Plastic Surgery and Burn Treatments
Blegdamsvej 9, 2100 Copenhagen
Phone: +45 35 45 33 53

Date: 10/05/22 Signature: 

Version 2.8
May 10, 2022

Trial synopsis

Title: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Lay title: Local Antibiotics for Women Undergoing Breast Reconstruction Surgery with Implants

Acronym: The BREAST-AB Trial

Trial design: A multi-center, investigator initiated, 1:1 randomized, double blind, placebo-controlled trial

Intervention: Application of gentamicin, vancomycin and cefazolin in a saline solution onto the implant and the dissected breast pocket used for breast reconstructive surgery

Objective: To determine the efficacy of local antibiotics in decreasing all-cause implant explantation

Inclusion criteria: Age ≥ 18, female, signed informed consent, breast reconstruction with implants including immediate/delayed reconstructions, bilateral/unilateral reconstructions and with or without flap reconstruction

Exclusion criteria: Pregnancy, breast feeding, known allergy towards any of the applied antibiotics, known anaphylactic reaction towards the same class of antibiotics as used in the trial, known allergy towards neomycin, known impaired renal function with GFR < 60 ml/min, participation in investigational drug trials and projects concerning disinfecting agents in the implant pocket and myasthenia gravis disease

Primary outcome: All-cause explantation of the breast implant within 180 days after the breast reconstruction surgery

Secondary outcomes:

- Time to explantation (days)
- All-cause explantation of the breast implant within 1 year after the breast reconstruction surgery (Y/N)
- Revision surgery with incision of the fibrous capsule within 180 days after the breast reconstruction surgery (Y/N)
- Revision surgery with incision of the fibrous capsule within 180 days after the breast reconstruction surgery (Y/N)
- Exchange of permanent implant to expander implant within 180 days after the breast reconstruction surgery (Y/N)
- Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction surgery (Y/N)

Tertiary outcomes: Assessed for patients undergoing unilateral breast reconstruction

- Time from surgery to discharge (days)

- Re-admission within 180 days after the surgery (Y/N)

Long-term outcomes: All-cause incision of the fibrous capsule and capsular contracture after 5, 10 and 15 years

Sample size: A total number of 1274 breasts undergoing breast reconstruction will be included in the trial. Assuming that 27 % of the patients undergo bilateral breast reconstruction, this entails 1003 included patients

Trial duration: 4 years and 180 days

Randomization: Stratified randomization according to the following factors:

- Unilateral or bilateral reconstruction
- Immediate or delayed reconstruction
- Previous or scheduled radiotherapy within the follow-up period (yes/no)

All patients undergoing unilateral breast reconstruction will be randomized to the trial drug or placebo in a ratio of 1:1. All patients undergoing bilateral reconstruction will be randomized to the trial treatment on one of their breasts and placebo to the contralateral breast. Combining these factors gives a total of 14 randomization strata per trial site. An allocation sequence will be made for each stratum and assign treatment in a fixed block size of two to ensure that the investigational drug and placebo is evenly distributed within each stratum

Treatment: The intervention treatment will consist of 1000 mg vancomycin (bactocin), 2 mL of 40 mg/mL gentamicin (hexamycin) and 1000 mg cefazolin (cefazolin "MIP") in a 500 mL sterile isotonic (9 %) saline solution. The placebo solution will consist of 500 mL of sterile isotonic (9%) saline. During the surgery, the responsible nurse will draw three 50 ml syringes from the infusion bag (in total 150 ml) and use it to wash the dissected implant pocket. Another 50 ml syringe will be drawn from the same infusion bag and used to wash the implant with the assigned solution prior to insertion in the implant pocket

Clinical follow-up: The included patients will adhere to the standard follow-up program according to the guidelines of the local treatment site

Blinding: The patients, surgeons and data assessors will be blinded to the treatment allocation throughout the trial period. The coordinating sponsor-investigator will be responsible for monitoring adverse events. The trial coordinating unit will have access to the randomization sequences. They will not take part in any treatment of the participants or analysis of data. In the case of emergency unblinding the trial coordinating unit can always be contacted

Safety: Treatment-related adverse events will be reported and assessed continuously throughout the trial period

Version 2.8
May 10, 2022

Trial Steering Committee

Thomas Bjarnsholt, Civil Engineer, DMSc, PhD
Professor at the Department of Microbiology, Copenhagen University Hospital, Rigshospitalet and Head of Costerton Biofilm Centre, SUND, University of Copenhagen

Tine Engberg Damsgaard, MD, PhD
Professor at the Department of Plastic Surgery and Burns Treatment,
Copenhagen University Hospital, Rigshospitalet

Peter Viktor Vester-Glowinski, MD, PhD
Medical Doctor and post doc at the Department of Plastic Surgery and Burns Treatment,
Copenhagen University Hospital, Rigshospitalet

Mikkel Herly, MD
Medical Doctor at the Department of Plastic Surgery and Burns Treatment,
Copenhagen University Hospital, Rigshospitalet

Søren J Sørensen, Cand. Scient., PhD
Professor and Head of the Section of Microbiology, Institute of Biology,
University of Copenhagen

Trial Coordinating Unit

Mathilde Nejrup Hemmingsen, MD
Department of Plastic Surgery and Burns Treatment
Copenhagen University Hospital, Rigshospitalet

Andreas Larsen, BMSc
Department of Plastic Surgery and Burns Treatment
Copenhagen University Hospital, Rigshospitalet

Tim Kongsmark Weltz, BMSc
Department of Plastic Surgery and Burns Treatment
Copenhagen University Hospital, Rigshospitalet

Version 2.8
May 10, 2022

Data Assessment Committee

Mathias Ørholt, MD
Department of Plastic Surgery and Burns Treatment
Copenhagen University Hospital, Rigshospitalet

Sebastian Wiberg, MD
Department of Anesthesiology and Intensive Care
Zealand University Hospital, Køge

An independent biostatistician who will be appointed before the assessment committee receives the data.

Version 2.8
May 10, 2022

Site Investigators	Clinical Trial Site
Lena Felicia Carstensen, MD	Department of Plastic Surgery, South-West Jutland Hospital, Finsensgade 35, 6700 Esbjerg +45 20 15 20 19
Mikkel Herly, MD	Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, +45 40 43 63 77
Lisbet Rosenkrantz Hölmich, Professor, MD, DMSc	Department of Plastic Surgery, Herlev and Gentofte Hospital, Borgmester Ib Juuls Vej 1, 2730 Herlev, +45 27 20 00 14
Vibeke Koudahl, MD, PhD	Department of Plastic Surgery, Hospital Little Belt, Kabbeltoft 25, 7100 Vejle +45 79 40 91 65
Volker-Jürgen Schmidt, Ass. Professor, MD	Department of Plastic Surgery, Zealand University Hospital, Sygehusvej 10, 4000 Roskilde
Camilla Bille, MD, PhD	Department of Plastic Surgery, Odense University Hospital, J. B. Winsløws Vej 4, 5000 Odense +45 65 41 24 45

Pharmaceutical manufacturer

Vancomycin
Bactocin®
MIP Pharma GmbH
Kirkeler Strasse 41
D-66 440 Blieskastel
Tyskland
Tlf: (+49) 68 42 96 09 0
Fax: (+49) 68 42 96 09 35
Email: info@mip-pharma.de
Website: www.mip-pharma.de

Cefazolin
Cefazolin "MIP"
MIP Pharma GmbH
Kirkeler Strasse 41
D-66 440 Blieskastel
Tyskland
Tlf: (+49) 68 42 96 09 0
Fax: (+49) 68 42 96 09 35
Email: info@mip-pharma.de
Website: www.mip-pharma.de

Gentamicin
Hexamycin
Sandoz A/S
Edvard Thomsens Vej 14
2300 København S
Tlf: 63951000
Fax: 63951001
Email: info.danmark@sandoz.com
Website: www.sandoz.dk

Natriumklorid
Natriumklorid "Fresenius Kabi" 9 mg/ml
Fresenius Kabi Denmark
Islands Brygge 57
2300 København S
Tlf: 33181600
Fax: 33181614
Emailadresse: info-dk@fresenius-kabi.com
Website: www.fresenius-kabi.com

Version 2.8
May 10, 2022

Table of contents

Version 2.8
May 10, 2022

1. Introduction

The incidence of breast cancer in Danish women is approximately 4700 per year.[1] Many women choose to undergo breast reconstruction with an implant following a mastectomy.[2] Unfortunately, implant-based breast reconstructions are associated with high complication rates. Postoperative infection of the breast and implant is one of the most severe short-term complications affecting around 5-10 % of the women.[3–7] Clinically infected implants must be surgically removed and the recovery period that follows is long and agonizing for the women. Subsequent attempts to reconstruct the breast are often postponed for several months or abandoned altogether.

Many strategies to prevent complications associated with bacterial contamination of the breast implant have been attempted.[8–13] According to a survey made by the American Society of Plastic Surgeons, the most widely followed approach is to apply antibiotics directly on the breast implant and the dissected tissue pocket to eliminate bacterial contamination during the surgery.[14] Although the use of local antibiotics on breast implants is now widespread, the treatment regimen has never been investigated in a randomized controlled trial.[15] This protocol will describe a randomized controlled trial that will investigate the effect of antibiotics applied locally on the implant and in the breast implant pocket on the incidence of infection that leads to explantation of the implant. The protocol has been designed in accordance with the SPIRIT 2013 Statement guidelines for protocol content.[16]

1.1. Bacterial contamination of the breast implant

The most prevalent bacterial agents associated with breast implant infections are similar to those of the breast duct and skin flora which suggests that these are possible sources of contamination.[17] The most common microorganisms found on infected breast implants are *Staphylococcus epidermidis* and *Cutibacterium acnes* (previously known as *Propionibacterium acnes*). Other bacteria that have been identified on breast implants are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.[18,19]

Previous studies propose that bacterial colonization of breast implants sometimes occur without any immediate clinical manifestations.[20,21] Instead, bacteria form a chronic, subclinical infection which is suspected to play a pivotal role in the development of a protracted immune reaction to the implant known as capsular contracture, affecting approximately 10-20 % of the patients.[22,23]

1.2. Local administered antibiotics

Local administration of antibiotics can achieve a high local concentration with a low systemic uptake[24] and thereby, minimize the systemic side effects while achieving high antibiotic penetrance. A high local concentration can ensure optimal effect of the antibiotics at the surgical site,[25] and thereby decrease the rate of postoperative surgical site infections, while potentially minimize the risk of antibiotic resistance. Local antibiotics also have the benefit of being independent from the tissue vascularization to achieve peak concentration as opposed to systemic antibiotics, which is an advantage during larger surgeries where the vascularization can be compromised.[25]

Studies have shown that the concentration of locally applied antibiotics in the surgical drain output is high during the first 24 hours[24] and after 72 hours, the concentration is negligible. Therefore, it is assumed that the potential side effects to the medication will occur within the first 72 hours and previous studies have not reported side effects to the local treatment.[26]

In 2001, Adams et al recommended an antibiotic regimen consisting of gentamicin, cefazolin combined with either bacitracin or vancomycin.[27] Internationally, this irrigation regimen has become the most commonly used for local breast pocket and implant irrigation.[28] In this trial we will investigate the combinations of gentamicin, cefazolin and vancomycin for irrigation for the breast pocket and breast implant.

1.3. Pre-clinical data

Preclinical data from in vitro models suggest that the combination of Gentamicin, Cefazolin and Vancomycin is the most efficient treatment against the bacterial species most commonly associated with breast implants.[27,29] Animal studies suggest that the local application of these antibiotics is safe.[30–33]

1.4. Clinical data

Current Evidence – a systematic review

The regimen of local antibiotics for breast implants and the dissected implant pocket has been widely applied in humans[14], but few studies have investigated the clinical effect. In May 2020, we searched scientific literature databases including Embase, Cochrane, Pubmed and Web of Science. We used the following search terms (((breast) AND (implant OR expander OR augmentation OR reconstruction)) AND (irrigation OR antibiotics OR antibacterial OR antiinfective OR antimicrobial OR disinfection OR bacitracin OR gentamicin OR vancomycin OR cefazolin OR neomycin)) AND (infection OR "capsular contracture" OR "capsular contraction" OR capsulitis). We included studies and reviews investigating the effect of any local antibiotics for irrigation of the implant and/or implant pocket in women undergoing implant-based breast reconstruction or cosmetic breast augmentation. Studies that did not list outcomes that were relevant for the primary and secondary outcomes of the BREAST-AB trial were excluded. The search identified 1697 studies of which 17 studies were included after title/abstract and full text screening. Seven review articles,[15,34–39] two prospective studies[40,41] and eight retrospective studies[41–49] were identified. No randomized controlled trials were identified. Most of the included studies included solely reported on patients undergoing cosmetic augmentation. Two studies included patients undergoing cosmetic breast augmentation and breast reconstruction, but they did not stratify the outcome.[48,49]

Two studies found a significant decrease in the infection rate when applying local antibiotics compared to a control group,[42,43] whereas one study found no significant decrease.[44] These studies were limited by the relatively small study populations and a poorly defined outcome and none of the studies were blinded. The rate of capsular contracture was found to be significantly decreased in two studies,[44,45] two studies found no significant decrease,[43,46] whereas one study found a significant increase in the capsular contracture rate.[41] However, all studies investigating

capsular contracture were limited by a short follow-up period for this long-term outcome. No adverse events have been reported in any of the included studies, and the local antibiotics were generally considered well-tolerated. See appendix 1 for a table of characteristics of the included studies.

1.5. Rationale

Administration of antibiotics directly on to the breast implant and dissected implant pocket will give a high concentration of the antibiotics where they are needed which may prevent bacterial contamination of the implant. This may decrease the rate of postoperative infections that lead to explantation of the implant and thereby improve the outcome for the patients.

1.6. Hypothesis

Local administration of gentamicin, cefazolin and vancomycin on the breast implant will decrease the rate of postoperative clinical infections compared to placebo.

2. Experimental design

2.1. Trial design

This trial is an investigator-initiated, randomized, double-blind and placebo-controlled clinical phase III trial. The triple antibiotic solution or placebo solution will be applied directly onto the implant used for breast reconstruction and the implant pocket. The included subjects who undergo bilateral reconstruction will be randomized to the triple antibiotic solution to one of their breasts and placebo to the contralateral breast. Those who undergo unilateral reconstruction will be randomized to the triple antibiotic solution or the placebo solution. See 4.2 for a more detailed description of the randomization. The triple antibiotic solution will consist of 1 g Vancomycin, 1 g Cefazolin and 80 mg Gentamicin diluted in 500 mL of saline.[27] The placebo solution will consist of 500 mL of saline. See section 5 for more information on the trial treatment.

2.2. Outcomes

2.2.1. Primary outcome

All-cause explantation of the breast implant within 180 days after the breast reconstruction surgery

Definition

All-cause explantation will be defined as explantation and discarding of the implant. Replacement of an expander with a permanent implant and replacement of a permanent breast implant with a new permanent breast implant due to cosmetic revisions such as asymmetry, implant malposition, change of size or implant rotation will not be counted as an explantation.

Rationale

The rationale for applying local antibiotics is to decrease the risk of severe complications associated with the presence of bacteria such as deep surgical site infection that leads to

explantation and discarding of the implant. Postoperative infection that leads to explantation of the implant will sometimes occur simultaneously with other complications where the cause of explantation may be unclear. Therefore, all-cause explantation is a logical and meaningful primary outcome.

The reason for excluding from the primary outcome: explantations of permanent implants followed by replacements with a new permanent implant for cosmetic reasons, is that such revisional surgery is not considered a proxy for severe complications that may be associated with a deep surgical site infection.

2.2.2. Secondary outcomes

The secondary outcomes will include:

- Time to explantation (days)
- All-cause explantation of the breast implant within 1 year after the breast reconstruction surgery (Y/N)
- Revision surgery with incision of the fibrous capsule within 180 days after the breast reconstruction surgery (Y/N)
- Exchange of permanent implant to expander implant within 180 days after the breast reconstruction surgery (Y/N)
- Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction surgery (Y/N)

Definition

Time to explantation will be defined as the amount of days between the breast reconstruction and the implant explantation surgery. The breast reconstruction surgery will be defined as the surgery where they received the allocated treatment. Surgical site infection will be defined according to the CDC classification of surgical site infetion[50] leading to antibiotic treatment with oral or intravenous antibiotics administered after the surgery.

Rationale

Time to explantation is important to determine the relation to the breast reconstruction surgery and the etiology of the event that leads to explantation of the implant. In some cases, the reconstructed breast may be upheld despite complications associated with bacteria by revisional surgery and therefore revisional surgery is an outcome of importance. Local antibiotics may decrease the incidence of postoperative surgical site infection requiring antibiotic treatment. Postoperative swelling and redness are to be expected after a larger surgery and can be difficult to distinguish from signs of infection. Therefore, surgical site infection that leads to antibiotic treatment is a logical outcome.

2.2.3. Tertiary outcomes

The tertiary outcomes will be assessed for patients undergoing unilateral breast reconstruction. The tertiary outcomes will include:

- Time from the breast reconstruction surgery to discharge (days)
- Re-admission within 180 days after the breast reconstruction surgery (Y/N)

Definition

Time to discharge will be defined as the amount of days between the breast reconstruction and the day of discharge. The breast reconstruction surgery will be defined as the surgery where the patient received the allocated treatment.

Rationale

The rationale for excluding bilateral patients from the tertiary outcomes is that all bilateral patients will receive placebo on one breast and the intervention on the contralateral breast. Therefore, patient related outcomes are only applicable for patients who undergo unilateral breast reconstruction. Postoperative infection that occurs during the hospital admission can prolong the admission period. Application of local antibiotics may shorten the admission period by decreasing the rate of postoperative infection occurring during the hospital admission. Infection that occurs after discharge can cause re-admission to the hospital. Local antibiotics may decrease the infection rate after discharge that require hospitalization.

2.2.3 Additional follow-up

The trial will include additional long-term outcomes focused on all-cause incision of the fibrous capsule around the breast implant, capsular contracture, Baker classification[51] and quality-of-life. See Gantt chart figure 1.

Definition

Capsular contracture and the Baker classification grade will be obtained from the National Patient Registry and the patients' medical journals after 5, 10 and 15 years. The BREAST-Q questionnaire[52] will be used to assess patient-reported outcomes. The patients will be contacted and asked to fill out the questionnaire with 5 year-intervals after the surgery.

Rationale

Previous studies suggest that bacterial contamination of the breast implant can occur without immediate clinical manifestation.[20,21] Instead, the bacteria form a chronic, subclinical infection which is suspected to play a pivotal role in the development of a protracted immune reaction to the implant called capsular contracture, affection 10-20 % of the patients.[22,23] The use of local antibiotics could potentially decrease the rate of capsular contracture by minimizing the bacterial contamination of the implant. Therefore, capsular contracture is a meaningful long-term outcome.

The application of local antibiotics may decrease the risk of postoperative complications and thereby decrease the risk of undergoing revision surgery. This in turn may lead to improved patient satisfaction and quality of life. Breast-Q is a validated tool used to quantify patient satisfaction and health-related quality of life after breast reconstruction surgery.[52]

Patients may be included in additional exploratory substudies at the time of implant explantation (e.g. expander removal). The exploratory substudies will be applied for in separate protocols to be approved by the relevant authorities and they will not interfere with this trial.

2.3. Setting and locations

The trial will be a nationwide multi-center trial with enrollment of patients from the following Danish hospitals:

- Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen
- Department of Plastic Surgery, Herlev and Gentofte Hospital, Borgmester Ib Juuls Vej 1, 2730 Herlev
- Department of Plastic Surgery, Zealand University Hospital, Sygehusvej 10, 4000 Roskilde
- Department of Plastic Surgery, Odense University hospital, J. B. Winsløws Vej 4, 5000 Odense
- Department of Plastic Surgery, South-West Jutland Hospital, Finsensgade 35, 6700 Esbjerg
- Department of Plastic Surgery, Hospital Little Belt, Kabbeltoft 25, 7100 Vejle

All sites have clinical experience and expertise in performing implant-based breast reconstructions.

2.4. Number of Subjects

The trial will include patients until a total number of 1274 breast reconstructions according to our power calculation. We estimate that this number will be distributed on approximately 1003 patients provided that approximately 27% of patients undergo bilateral procedures. A total of 637 breasts will be allocated to placebo and 637 breasts will be allocated to treatment with the local antibiotic solution. The statistical considerations behind the sample size calculation is elaborated in section 8.1.

2.5. Trial Duration

We plan to begin inclusion in January 2021 at Rigshospitalet and Herlev Hospital. The other trial sites will begin enrollment thereafter according to the plan outlined below. We expect to begin inclusion at Zealand- and Odense University Hospital in spring 2022 followed by South-West Jutland Hospital and Hospital Little Belt in the autumn 2022. Additional trial sites may be applied for during the trial period if we do not meet our expected aim for included patients. See Gantt chart in figure 1.

We expect to include the 1003 patients over a 4-year period with planned completion January 2025, hence the last follow-up after 180 days will be completed after in July 2025. Currently, 700 women undergo reconstruction with implants each year in Denmark.[53] Therefore, we assume that it will be feasible to include approximately 334 patients per year.

Trial site	Inclusion period	Expected no. of included patients
Rigshospitalet	Jan 2021 – Jan 2025	274
Herlev Hospital	Jan 2021 – Jan 2025	253
University Hospital Zealand	Feb 2022 – Jan 2025	162
Odense University Hospital	April 2022 – Jan 2025	162
South-West Jutland Hospital	Oct 2022 – Jan 2025	76
Hospital Little Belt	Oct 2022 – Jan 2025	76
Total		**1003**

3. Subjects eligibility

All trial candidates will be evaluated for suitability by a medical doctor with expertise in the field of breast reconstruction surgery. All potential participating patients will receive oral information by the medical doctor and all information material will be given to the patient before the written informed consent form is signed. See participant timeline in figure 2.

3.1. Inclusion criteria

The patients must fulfill all the following criteria to be eligible for inclusion in the trial:

- Age ≥ 18 years
- Biologically female
- Signed informed consent
- Scheduled for breast reconstruction with implants or expanders including:
    a. Immediate or delayed reconstructions
    b. Bilateral or unilateral reconstructions
    c. With or without simultaneous flap reconstruction

3.2. Exclusion criteria

Patients are considered ineligible if any of the following criteria is fulfilled:

- Pregnancy
- Breast feeding
- Known allergy towards Vancomycin, Gentamicin and Cefazolin
- Known anaphylactic reaction towards other beta-lactam antibiotics or aminoglycosides
- Known allergy towards neomycin
- Known impaired renal function with GFR < 60 mL/min
- Participation in investigational drug trials and projects concerning disinfecting agents in the breast implant cavity
- Myasthenia Gravis

3.3. Pregnancy

Fertile women with child-bearing potential must provide a negative urine HCG prior to inclusion in the trial.

4. Enrollment

The patients will be registered in the trial after providing written consent (via the written consent form or a digital signature). The registered patients are considered enrolled in the trial when they have received the treatment. Each step of the enrollment procedure is described below.

4.1. Registration

All patients scheduled for a preoperative visit concerning a breast reconstruction procedure will be screened for eligibility and recorded in the individual trial site's screening log (appendix 2). No personal data will be recorded in the screening log. The following variables will be registered in the screening: screening number, screening date, initials of the person conducting the screening, age of the patient, if available date of pre-operative visit and surgery, eligibility of the patient yes/no and if "no", reason for non-eligibility. All patients who are considered eligible and have provided a written consent will be registered in the trial with a letter code for each site (e.g. RH for Rigshospitalet) combined with a record ID. The record ID will be assigned in sequential order as subjects are registered (1, 2, 3). Registration will include date of registration, central registration number, unilateral or bilateral reconstruction, type of surgery (immediate or delayed reconstruction) and radiotherapy status. The identification number remains constant throughout the trial.

4.2. Randomization and treatment assignment

Registered subjects will be randomized to placebo or the trial drugs on the day of surgery or the day before, and they will be considered enrolled in the trial when they have received the trial treatment. The randomization number will be the same as the record identification number. All patients undergoing unilateral breast reconstruction will be randomized to the trial drug or placebo in a ratio of 1:1. All patients undergoing bilateral reconstruction will be randomized to the trial treatment on one of their breasts and placebo to the contralateral breast (Investigational Product Dosage and Administration, section 5). See figure 3.

We will use a stratified randomization to ensure that potential risk factors which could confound the outcome are evenly distributed in the placebo and intervention group. The randomization strata will be generated by the following factors:

- Unilateral or bilateral reconstruction
- Immediate or delayed reconstruction
- Previous radiotherapy and/or planned radiotherapy within the follow-up period (yes/no)

When the three factors are combined, we get a total of 14 randomization strata per trial site. See appendix 3 for an overview of the 14 randomization strata. An allocation sequence will be made for each stratum and assign treatment in a fixed block size of two to ensure that the investigational drug and placebo is evenly distributed within each stratum. The fixed block size of two will not increase the risk of the investigator anticipating the allocation because the investigators are blinded to the treatment throughout the trial.

A member of the trial coordinating unit will access the computer-generated allocation sequence via RedCap The allocation will be registered in a REDCap module only available for members of the trial coordinating unit, who are unblinded.

4.2.1. Treatment assignment in two-stage breast reconstruction

Most implant-based breast reconstructions are performed in a single surgery with a permanent breast implant. During the surgery, the surgeon evaluates the quality and vitality of the dissected skin flaps before inserting the breast implant. In some patients, the skin quality is not considered suitable to allow for insertion of the permanent implant. These patients will be reconstructed in two stages, where an expander implant is used in the first surgery. The expander implant is used to expand the tissue before it can be replaced with a permanent implant after approximately 3 to 9 months of expansion. During the first surgery where the expander implant is inserted, the patient will be assigned to treatment according the randomization stratum. The allocation sequence number will be registered. At the second surgery where the expander is replaced with the permanent implant, the same treatment allocation will be used (e.g., if the right breast received treatment with the antibiotic solution in the first surgery, the right breast will receive treatment with the antibiotic solution in the second surgery). See figure 3.

4.2.2. Treatment assignment if a unilateral patient later becomes bilateral

In some cases, a unilateral patient can switch to the bilateral set-up. An example could be that a patient develops unilateral breast cancer, undergo mastectomy and reconstruction and then later in the trial period decide to undergo prophylactic risk-reducing mastectomy and reconstruction of the other breast. In this case, the patient would be assigned to either placebo or the trial drug in the first surgery. Then, when the patient undergo the second prophylactic surgery on the other breast, she will be allocated to receive placebo, if she had received antibiotics in the first surgery and vice versa, if she had received placebo in the first surgery she would be allocated to antibiotics on the other breast in the second surgery.

4.3. Registration failures

Registered subjects who are ineligible for randomization will be recorded as screening failures and they will be registered along with the reason for exclusion.

4.4. Discontinuation from the trial

Patients who withdraw their informed consent at any point during the trial period will be omitted from the trial and registered as “withdrawal of consent”. The coordinating sponsor investigator may omit participants from the trial at any point during the trial period due to safety of the participant. There will be no additional follow-up or data collection from these patients. The trial treatment is administered as a single dosage and therefore, exclusion will not have any effect on the trial treatment. The data analysis in the end of the trial will be performed on a modified intention-to-treat population, defined as all randomized patients with a valid informed consent.

4.4.1. Registration of dropouts

A designated representative at each trial site will be responsible for contacting the trial coordinating unit in case an included subject withdraws consent or is unable to complete the follow-up. All excluded patients will be recorded including date, central registration number, reason for exclusion and treatment allocation. Patients excluded from the trial will continue to follow the scheduled follow-up visits as a part of the standard treatment. Exclusion from the trial will not interfere with the standard treatment or entail any additional procedures or follow-up visits. Dropout rates will be monitored continuously by the sponsor-investigator.

4.4.2. Replacement of dropouts

Patients who drop out of the trial after enrollment (allocation to trial treatment) will not be replaced. The sample size calculation accounts for a drop-out rate of 5 %. In case of a drop-out rate of more than 5 %, we will apply imputations by chained equations and repeat the primary analysis (for the primary outcome) after imputations.

5. Treatment procedures

The trial drug will be administered as a single dosage during the breast reconstructive surgery. The administration procedure will be identical for the antibiotic solution and placebo. Only qualified healthcare personnel will perform the administration of trial drugs and no self-administration will take place. All personnel that handles investigational products will be instructed by members of the trial coordinating unit.

5.1. Investigational drugs

Gentamicin: 40 mg/ml gentamicin sulfate, 2 mL suspension in glass ampoules containing clear, colorless suspension without visible particles. Gentamicin is a broad-spectrum, bactericidal aminoglycoside primarily targeting gram-negative rods. Gentamicin (Hexamycin) produced by Sandoz A/S can be used but other producers of the same drug may be used as an alternative.

Cefazolin: 2096,72 mg cefazolin natrium equivalent to 2000 mg cefazolin of white or almost white powder in a capped vial. Cefazolin is a bactericidal antibiotic targeting both gram-negative and gram-positive bacteria. Cefazolin produced by MIP Pharma GmbH can be used but other producers of the same drug may be used as an alternative.

Vancomycin: one capped vial contains vancomycin hydrochloride equivalent to 1000 mg vancomycin as a finely grounded white powder with a pink to brown nuance. Vancomycin is bactericidal and targets gram-positive bacteria. Vancomycin (Bactocin®) produced by MIP Pharma GmbH can be used but other producers of the same drug may be used as an alternative.

All investigational drugs will be purchased through each trial site's clinical pharmaceutical services and their respective purchasing agreements. The investigational drugs will be kept and prepared in accordance with the manufacturer's recommendations (see 'summery of product characteristics' for cefazolin, gentamicin and vancomycin. The investigational drugs are part of the standard drug selection available at all trial sites. Therefore, we will not account for the overall stock of medicine.

However, we will account for the use of investigational medicine for each patient enrolled in the trial, including batch-number, expiration date, patient registration number and date of administration.

5.2. Preparation of the drug solutions

The preparation of the trial drug solution will take place in a medication room on the trial site on the day of the surgery or day before the surgery. A designated trained nurse will be responsible for the preparation and labelling of the trial drug solution. To minimize errors, it will be double checked. The labelling will include the trial identification number and marking of which breast the treatment will be applied to (right or left). The manufacturing nurse will contact the trial coordinating unit to confirm his/her identity as investigational drug manufacturer and obtain instruction as to how to allocate the trial treatment. The communication between the Trial coordinating unit and the manufacturing nurse will be looped to prevent mistakes regarding the allocation. A designated person will deliver the prepared solutions to the operation room, and the surgeon and the scrub nurse will be blinded for the allocation. A local SOP describing the preparation of the trial drugs will be compiled for each trial site (See appendix 4 for the SOP).

5.2.1. The antibiotic solution

The antibiotic solution will contain 1000 mg vancomycin, 80 mg gentamicin and 1000 mg cefazolin. Two syringes of 20 mL sterile saline will be drawn from an infusion bag containing 500 mL of sterile isotonic (9%) saline. The drawn saline will be infused in the capped vials containing cefazolin and vancomycin to dissolve the powder. The entire content of the vial containing vancomycin (20 mL) will be drawn back into the syringe and reinfused in the infusion bag. Only 10 mL of the cefazolin solution will be drawn from the capped vial containing the dissolved cefazolin. The 10 mL will also be reinfused in the infusion bag. The remaining content the capped vial will be discarded as medical waste. Hereafter, the 2 mL gentamicin suspension will be drawn into a syringe and infused in the infusion bag already containing vancomycin and cefazolin. See figure 4 for an illustration of the treatment preparation.

5.2.2. The placebo solution

The placebo solution will consist of 500 mL of sterile isotonic (9%) saline contained in a similar infusion bag.

Both the antibiotic solution and the placebo solution will be achromatic and will be indistinguishable from one another to ensure blinding of the health care personnel administering the drugs.

5.3. Investigational product administration

The assigned solution will be administered in an enclosed infusion bag. During the surgery, the responsible nurse will draw three 50 ml syringes from the infusion bag (entailing 150 ml) and use it to wash the dissected implant pocket. Another 50 ml will be drawn from the same infusion bag and used to wash the implant with the assigned solution prior to insertion in the implant pocket. The rest of the content in the infusion bag will be discarded as medical waste. Patients undergoing unilateral breast reconstruction will receive the assigned solution in one infusion bag marked either left or right. The patients undergoing bilateral breast reconstruction will be assigned to the

antibiotic solution to one breast and placebo solution to the contralateral breast, and the allocation sequence will determine which breast (right or left) gets which solution. The assigned solution bags will be marked right and left. See appendix 5 for SOP.

5.4. Dosage adjustments

The dose of investigational products will be fixed. The drug will be administered as a single dose, and one time only. No continuous administration will occur. It will not be possible to adjust the treatment after the administration of the treatment.

5.5. Concurrent medication

All included patients will be treated according to the local guidelines for breast reconstruction surgery at each trial site. The trial intervention will not interfere with any treatment procedures or administration of medication.

5.6. Blinding

The trial will double-blind so that the patients, site investigators and data monitors will be blinded to the allocation. Only the designated nurses and the members of the trial coordinating unit (who provide the randomization number and treatment allocation) are not blinded to the allocation. The unblinded persons will not in any way be part of the treatment, clinical evaluation of outcomes or data assessment.

The intervention drug will be prepared in infusion bags that will be indistinguishable to the placebo solution (infusion bags with saline). Both solutions are colorless and identical in appearance without any identifying features, and therefore we do not anticipate any risk of unintentional unblinding. The designated manufacturing nurse will contact the trial coordinating unit to receive the allocation by telephone. The randomization number and the treatment allocation will be provided by the trial coordinating unit. An emergency telephone number to the trial coordinating unit will be available to access the treatment allocation of individual patients in the case where emergency unblinding is necessary. If unblinding should occur, it will be documented via the case report form. The unblinded patient will not be excluded from the trial.

The patients will remain blinded until the end of the additional follow-up period. The rationale for keeping the patients blinded is to minimize bias when assessing the long-term outcomes. The patients can be unblinded upon request if they withdraw their consent to participate in the trial.

5.6.1. Ensuring blinding

The randomization number will ensure that the allocation is given during both surgeries in situations where the intervention treatment is repeated, for instance in two stage breast reconstruction (see section 4.2.1.) and if a unilateral patient becomes bilateral (see section 4.2.2.). During the first surgery, the registration number which equals the randomization number will be registered in the case report form. During the second surgery, the manufacturing nurse will contact the trial coordinating unit to obtain the treatment allocation and members of the trial

coordinating unit (who are unblinded) will look up the patient and inform the manufacturing nurse of the treatment allocation. The manufacturing nurse will not take part in any treatment procedures.

6. Data collection

The site investigators, along with members of the trial coordination unit, will be responsible for trial related data collection and entry. The individual trial site investigator may delegate assignments to designated doctors, scholarship students or nurses registered in the site-specific delegation log. Limited trial specific data will be entered into a numbered case report form (see appendix 6) at the time of inclusion and the surgery. This along with the screening log will be the only source data and all additional data will be obtained from the patients' medical records. Data will be entered directly into REDCap.

6.1. Variables

All enrolled patients (i.e. patients who have been assigned to treatment) will be entered into the database. An overview of included variables is provided below.

6.1.1. Pre-surgery variables

Trial related variables

- Study ID
- Site (location)
- Unilateral or bilateral breast reconstruction
- Immediate or delayed breast reconstruction
- Prophylactic or cancer (including carcinoma in situ)
- Radiation therapy status (Y/N)
- Name
- CPR number
- Date and name of data collector

Patient demographics

- Height (cm)
- Weight (kg)
- Smoking (never, former, active)
- Alcohol consumption (units per week)
- ASA classification (class I-VI)
- Race

Comorbidities

Prescription medications

Oral or intravenous antibiotic treatment within 2 months up to the surgery (Y/N, name and dose of antibiotic)

Prior breast surgery (Y/N, type of surgery, date)

Radiation therapy (dose and fraction)

Chemotherapy (type, dose, duration, frequency and no of cycles)

Antihormonal therapy (type, dose and duration)

Antibody therapy (type, dose and duration)

6.1.2. Surgical variables

Trial related variables

Date and time of surgery

Randomization number

Direct-to-implant or expander breast reconstruction

Deviations from the protocol

Date and time of treatment administration

Date and name of the data collector

Surgery characteristics

Mesh (Y/N)

Drain (Y/N)

Operative time (hours, minutes)

Implant type (brand, volume, texture, serial no.)

Implant placement (prepectoral or subpectoral)

Type of mastectomy procedure (nipple sparring KHAC10 or skin sparring KHAC15)

Type of reconstruction (KHAE00, KHAE05)

Thickness of mastectomy flap (mm)

Pre- and perioperative medications

VAC (Y/N)

6.1.3. Post-surgery variables

Characteristics

- Date of discharge
- Time to drain removal
- Post-operative medication
- Hematoma (Y/N)
- Mastectomy flap necrosis (Y/N)
- Nipple-areola-complex necrosis (Y/N)
- Seroma (Y/N)
- Explantation (Y/N)
- Date of explantation
- Indication of explantation
- Surgical site infection (Y/N)
- Bacterial agent (culturing/PCR)
- Revisional surgery with incision of the fibrous capsule (Y/N)
- Date of revisional surgery
- Indication of revisional surgery
- Baker grading
- Local adverse event
  - Severity of event
  - Time of event
  - Treatment/action taken
- Exclusion/loss to follow-up
  - Reason for exclusion

6.2. Clinical follow-up

The included patients will adhere to the standard follow-up program according to the guidelines of the local treatment site. There will no trial specific clinical follow-up visits. The patients will be instructed to contact the local treatment site if they should experience adverse events after they have been discharged. Additionally, the patients will be instructed to contact us if they receive relevant treatment related to the reconstructed breast at another hospital than their primary treatment site or via their general practitioner within 180 days after the surgery.

6.3. Data quality and security

Each variable is clearly defined in the case report form. Each data field will be provided with a definition of the variable, category for categorial variables and units for continuous variables.

All relevant trial documents including the signed consent form for each patient will be stored in the trial master file in a secure, locked place at each individual site. Only the site investigator and designated personnel will have access. The trial has been approved by the Danish Data Protection Agency. The files will be stored for 25 years, after which they will be destroyed.

7. Assessments of safety and harm

Women receiving breast cancer treatment and subsequently undergo breast reconstructive surgery are in high risk of experiencing adverse events in relation with the surgical treatment and cancer. These adverse events will not be registered as trial drug-adverse events.

The trial drugs are widely used internationally, and the adverse reaction profile for each drug is well-defined. (See 'Summery of Product Characteristics' section 4.8 for each drug). Previous studies show, that only low levels of locally administered antibiotics enter the bloodstream and therefore, the systemic side effects to the trial drugs are expected to be negligible.[54]

7.1. Expected adverse events unrelated to the trial drug

The complication rate following breast reconstruction is relatively high, and certain adverse events are to be expected after surgical resection of the breast and reconstruction with an implant. Several postoperative adverse events are likely to occur in the included patients due to the surgery and patient comorbidities and these events will not be registered as adverse events related to the interventional drugs. The following adverse events are expected in the included patients and will not be registered as adverse events to the trial treatment:

- Surgery specific complications: surgical site infection, implant malposition, expander deflation, expander port malfunction, implant/expander exposure, implant/expander rupture, wound dehiscence, hematoma, flap necrosis, seroma, capsular contracture, nerve damage, pain and lymphedema.
- Infectious disease including sepsis without signs of surgical site infection and fever without signs of surgical site infection.
- Cardiovascular disease including stroke, acute myocardial infarction, heart failure, arrythmia, cardiac arrest, pulmonary embolism, DVT and coagulopathy.
- Gastrointestinal disease including diarrhea, nausea, vomiting, gastroenteritis, colitis and ileus.
- Adverse events related to the kidneys and urinary tract including uremia, proteinuria, hematuria, interstitial nephritis, upper and lower urinary tract infection and pre-renal and post-renal kidney failure.
- Liver- and biliary tract disease including increase in serum liver enzymes levels, increase in bilirubin and alkaline phosphatase, hepatitis, liver cirrhosis, cholecystitis and pancreatitis.

- Respiratory disease including dyspnea, pleural effusion, pneumonia, pharyngitis, sinusitis and rhinosinusitis.
- Neurological disease including seizures, dizziness, impaired vision and vertigo.
- Metabolic disease including hyperglycemia and hypoglycemia.
- Immunological disease including thrombocytopenia, leukopenia, granulocytopenia, agranulocytosis, pancytopenia, leukocytosis, granulocytosis, anemia and polycythemia.
- Psychiatric disease including depression.
- Cancer recurrence.

7.2. Adverse events possibly related to the trial drug

The presumed relation to the trial drug will be evaluated using the 'Summery of Product Characteristics' for Gentamicin, Cefazolin and Vancomycin.

Examples of events that are likely to be related to the trial drugs are:

Erythema multiforme

Urticaria

Angioneurotic edema

Toxic epidermal necrolysis

Steven Johnsons' syndrome

Anaphylactic shock

Red man syndrome (appearing after a maximum of 10 minutes after administration)

Acute tinnitus

Acute deafness

Drug induced-acute kidney injury

Myasthenia gravis-like syndrome

Local adverse events (incl. surgical site infection, skin irritation, erythema, delayed wound healing, itching)

7.3. Adverse Event Reporting

Local antibiotics therapy is considered safe.[24] Previous studies have shown that the serum level of antibiotics after local application is low,[26] and therefore the risk of systemic organ toxicity is low. Gentamicin, cefazolin and vancomycin have all been approved for marketing for many years and have a well-known systemic adverse reaction profile. The combination of gentamicin, vancomycin and cefazolin has been used for local breast pocket irrigation for many years and is considered

safe.[36] Due to the extremely low systemic uptake when using locally administered antibiotics, the event of systemic adverse events following the trial intervention is considered very unlikely.

Adverse events related to the study drug will be assessed continuously by the co-investigator at each study site during the admission period (typically 72 hours). The trial drugs have a short half-life period of maximum 6 hours and will only be administered one time during the surgery. Therefore, it is considered very unlikely that adverse events related to the trial drugs should occur after discharge. If the patients experience adverse events, the site investigator will be responsible for registering the adverse event directly in the case report form. The patients will be instructed to contact the local treatment site if they should experience adverse events between the scheduled follow-up visits. The site investigator will report all adverse events to the coordinating sponsor-investigator who will be responsible for monitoring the safety of the trial. Each adverse event will require the investigator to fill in the AE form including the following variables: patient identification number, description of event, onset and end of event, severity, relation to the intervention, action taken and outcome.

Any adverse events occurring during the trial will be treated and monitored according to the local guidelines.

7.3.1. Adverse Events (AE) and Adverse Reactions (AR)

Any event that occurs after administrations of the trial drug regardless of the relation to the trial drug will be defined as an adverse event. Adverse reactions will be defined as events that are related to the trial drug.

7.3.2. Serious Adverse Events (SAE) and Serious Adverse Reactions (SAR)

For each recording of adverse event, the event will be evaluated as to whether it was a serious adverse event. A SAE will be defined as an AE that is life threatening, results in death, requires prolonged hospitalization or results in significant disability.

The site investigator at each trial site will be responsible for contacting the coordinating sponsor-investigator in case of serious adverse events within 24 hours of awareness. The site investigator will record the event in the case report form.

All serious adverse reactions will be reported yearly to the Danish Medicines Agency and the Regional Ethics Committee by the sponsor-investigator during the study period of three years and 6 months. After last patient last visit, serious adverse events will no longer be reported annually to the Danish Medicines Agency and The Regional Ethics Committee, since the effect of the study drug is considered negligible after 30 hours, and it is unlikely that any serious adverse events related to the study drug would occur after three days.

During the long-term follow-up, serious adverse events will not be reported in the annual safety report (ASR). Serious adverse events will be registered in the trial and included in a final clinical study report after the last long-term follow-up. See Gantt chart figure 1.

7.3.3. Suspected Unexpected Serious Adverse Reactions (SUSARs)

A SUSAR is an unexpected and serious presumed reaction to the trial drug. Section 4.8 in the 'Summery of Product Characteristics' for each drug (cefazolin, gentamicin and vancomycin) will be used as the reference safety information to determine whether or not the serious adverse event is unexpected. The sponsor-investigator will be responsible for that all relevant information about SUSARS, which are fatal or life threatening, is recorded and reported to the Regional Health Ethics Committee and the Danish Health and Medicines Authority as soon as possible, and no later than 7 days after the sponsor-investigator has been informed of such an event. No later than 8 days after the reporting, the sponsor-investigator is responsible for informing the Regional Health Ethics Committee and the Danish Medicines Agency of relevant treatment initiated by the co-investigator or a doctor at the trial site. Any other SUSAR must be reported to the Regional Health Ethics Committee and the Danish Medicines Agency no longer than 15 days after the sponsor-investigator has been informed. All trial investigators will be informed by the coordinating sponsor-investigator in the event of a SUSAR. See Gantt chart figure 1.

7.3.4. Severity of Adverse Events

The severity of each adverse event suspected to be related to the trial drug will be graded accordingly

- Mild: transient symptoms, with no interference in normal daily activity
- Moderate: persistent symptoms, resulting in moderate inhibition of daily activity
- Severe: persistent symptoms, resulting in severe inhibition of daily activity

7.3.5. Relationship of AE to Trial Intervention

For each AE suspected to be related to the trial drug, the probability will be rated accordingly

- Probable: there is good reason and adequate documentation to assume causal relationship
- Possible: a causal relationship is likely and cannot be dismissed
- Unlikely: the event is most likely related to an etiology other than the intervention
- Unknown: causality is not assessable

7.3.6. Adverse Reactions reporting during long-term follow-up

The annual reporting of SAR will not apply during the long-term follow-up period. Serious adverse reactions that we learn of during the long-term follow-up period of 5, 10 and 15 years will be recorded and included in a final clinical study report after the last long-term follow up.

SUSARS will be reported continuously to the Danish Medical Agency throughout the long-term follow-up period. See Gantt chart in figure 1.

8. Statistical considerations

8.1. Sample size

The trial will be powered towards the primary outcome. Previous data[3] do not suggest that there is an overrepresentation of bilateral infections compared to the unilateral infection rate which suggests that infections may be randomly distributed. Therefore, we do not assume that infections are correlated between breasts within the same patient. However, due to the paired design in the patients that undergo bilateral reconstruction, any correlation between the individual patient's breasts will increase the statistical power of this trial. The independent sampling unit of this trial will be 'breast', and the trial will be powered towards 'number of breasts', so that the final number of included patients will depend on the proportion of patients undergoing bilateral breast reconstruction. The incidence of the primary outcome is reported at 10%. With an α-level of 0.05, the trial will have a power of 0.90 to detect an absolute risk reduction of 5% if 1158 'breasts' are included. We will include patients, until a total number of 1274 breasts have completed the follow-up period of 180 days to account for a dropout rate of 10%. We estimate that 1003 patients will be included in the trial if approximately 27% of the patients undergo bilateral breast reconstruction.

8.2. Statistical analysis plan

The statistical analyses will be conducted on a modified intention-to-treat population, defined as all randomized patients with a valid informed consent. Categorical variables will be presented as frequencies whereas normally distributed continuous variables will be presented as mean ± SD, and as median (25^{th} percentile-75^{th} percentile) if non-normally distributed. Differences in endpoints (including the primary outcome) between the treatment allocations will be analyzed with mixed effects models taking into account the correlation between breasts in patients undergoing bilateral surgery. The stratified randomization approach will ensure an even distribution of known outcome risk factors between the placebo group and the treatment group. Logistic regression models will be applied to compare the odds of the primary outcome between the two treatment groups. The models will be adjusted for potential confounders, including age, smoking, body mass index, indication for surgery (prophylactic mastectomy versus mastectomy after cancer diagnosis), immediate reconstruction versus delayed reconstruction, bilateral versus unilateral surgery, and radiotherapy status. In case of missingness greater than 5% for the primary outcome, we will apply multiple imputations by chained equations and repeat the primary analysis as sensitivity analysis. A statistical significance level of 0.05 will be applied throughout. The open source statistical program "R" (http://www.r-project.org) will be used for data treatment and statistical analysis.

8.3. Registration of changes

Changes to the original statistical analysis plan will be recorded in the sponsor's trial master file before unblinding.

9. Ethical considerations

This trial will be conducted in accordance with EU and national legislation on medical research in capable patient volunteers. Eligible subjects shall provide oral and written informed consent to participate in the trial. The informed consent can be withdrawn by the participant at any time during the trial after which the patient will convert to receiving treatment as determined by local guidelines. All data on trial participants will be protected according to the General Data Protection Regulation (GDPR), the data protection law and the Danish Health Act. The project is approved by the Danish Data Protection Agency and is to be approved by the Danish Medicines Agency and the Regional Committee on Health Research Ethics. The trial will be conducted according to national and international standards of good clinical practice (GCP) and will be monitored by the regional GCP unit.

9.1. Ethical justification

The trial will investigate the beneficial effects and potential side-effects of applying gentamicin, vancomycin and cefazolin locally onto the breast implant during breast reconstruction surgery. This may limit bacterial contamination of the implant and thereby decrease the risk of postoperative infection which is associated with a poor outcome for the patients. Therefore, participation in this trial could benefit the individual participant.

Inclusion in the trial may benefit the individual subject by decrease the risk of undergoing explantation of the implant, minimizing the risk of postoperative infection, minimize the hospitalization period and may as well improve the outcome for future women undergoing implant-based breast reconstructive surgery. Alternatively, we may find that the local antibiotics do not have a clinically relevant effect and perhaps negative side-effects that should be explored further.

The trial drug regimen is widely used internationally[14] but the potential positive and/or negative effects of using local antibiotics on breast implants have never been tested against placebo in a randomized trial. See appendix 1 for a review of the current literature.

Though the drug regimen has not been tested in a randomized controlled trial, the drugs have been applied by plastic surgeons for several years, and no adverse events has been reported.[42,44,48,55] Therefore, the drug regimen is expected to be of minimal risk to the subjects in the trial. Moreover, the systemic levels of antibiotics after locally applied antibiotics has been shown to be much lower than the levels seen with systemic antibiotics and the trial treatment consists of a single dose, and therefore we do not expect systemic adverse reactions to the trial drugs.[36]

Currently, no clinical guidelines exist in Denmark regarding the prophylactic use of local antibiotics to breast implants, and the treatment depends on the local approach at each hospital and the individual surgeon's preference. Allocation to placebo in this trial is therefore considered ethically acceptable. Application of local vancomycin on the breast implant has been used routinely at the Department of Plastic Surgery, Herlev and Gentofte Hospital, in the past years. This treatment regimen is not based on any evidence and is scientifically unjustified. Therefore, we find it ethically acceptable to include patients from Herlev and Gentofte Hospital in the trial. All eligible patients

from Herlev and Gentofte Hospital will receive this information before providing consent and specific written patient information material will be provided for these patients. Patients at Herlev and Gentofte Hospital who are not included in the trial will receive local vancomycin during the breast reconstruction surgery.

The results from this trial could change the guidelines for breast reconstruction surgery on an international level and be used to provide patients with evidence-based treatment.

9.2. Informed consent

Registered patients will receive information about the trial in their e-boks. They will be informed that the trial coordinating unit will contact them to provide oral information about the project after they have had time to read the information material about the trial. The contact information for registered patients will be passed on to the trial coordination unit from the treatment site.

Recruitment of trial participants will be carried out at the time of the preoperative patient visit or by telemedicine (i.e. telephone or a secure video connection) by a medical doctor with relevant expertise. All patients are encouraged to bring a third party (i.e. relative or partner) to this appointment. The responsible medical doctor can assign a designated nurse or medical student with relevant expertise to provide the patient with both oral and written information concerning the trial during this visit as the medical doctor has limited time available at the initial pre-operative consultation. The investigator carrying out the recruitment will be responsible for obtaining the informed consent (either by a digital consent or a written consent form) from the patients prior to any protocol-related activities. The conversation will take place in a private room behind a closed door. Participation in the trial will not influence the choice of treatment. The patient and the responsible medical doctor must personally provide a dated signature digitally or on a consent form. The informed consent can be withdrawn by the patient at any time and without explanation, after which the patient will receive the standard treatment according to the local guidelines. No personal data will be collected patients before informed consent is obtained.

9.2.1. General considerations

We will strive to provide the patients with at least 24 hours of consideration, but in some patients this will not be possible. For instance, when it comes to patients undergoing primary breast reconstruction, the breast reconstruction is performed during the same surgery as the cancer surgery and the patients are treated in an accelerated cancer treatment course. Due the short period of time from planning to carrying out the surgery, the decisions regarding possible use of implants or expander for reconstruction are often made a few hours before the surgery. In these cases, the patients will have at least 2 hours of consideration.

10. Direct access to source data/documentation

The site investigator will permit direct access to source data blinded for treatment allocation for monitoring, audits and reviews by the Health Ethics Committee, Good Clinical Practice unit, Danish Medicines Agency and other regulatory authorities.

11. Data management

All data management will be conducted according to guidelines of the Danish Data Protection Agency. The data will be kept in REDCap. The database will be maintained for 25 years from the last patient visit and anonymized when the approval from the Danish Data Protection Agency terminates.

12. Quality control and quality assurance

The trial will be conducted according to the approved protocol and will comply to standard procedures for quality control and quality assurance. The investigator at each trial site will report any deviations from standard protocol to the coordinating sponsor-investigator either by direct contact or via the case report form.

The trial conduct, data generation, data documentation and reporting will be in accordance with ICH-GCP guidelines and the trial will be monitored by the national Good Clinical Practice (GCP) unit. Monitoring will be conducted upon initiation of the trial, during the trial and at termination of the trial.

The sponsor-investigator, local trial site investigators and the trial coordinating unit are responsible for maintaining up-to-date accrual information, enrollment status and safety data. It is the responsibility of the sponsor-investigator to ensure oversight of trial related activities and on a yearly basis, report trial progression, enrollment status and safety data to the Trial Steering Committee (see Trial Steering Committee Charter). The sponsor-investigator will submit all relevant documents to the board members for scientific progress review. The sponsor-investigator is responsible for ensuring that access to clinical trial data is consistent with data protection principles and in accordance with the patients' informed consent provided in relation to their participation in the clinical trial.

The trial steering committee will be responsible for data monitoring and quality control of the data extracted from the patients' medical journals at the long-term follow-up after 5, 10 and 15 years.

13. Finance and insurance

The trial is funded by a project grant in surgical research of 2,975,000 DKK from the Novo Nordisk Foundation. The grant budget will cover a PhD salary, a part-time post-doc salary, and medicine and materials required for the trial. See attached research budget. The grant is disbursed to Professor Tine Engberg Damsgaard on behalf of Rigshospitalet and administered by the Financial Department of the Centre of Head and Orthopedics, Rigshospitalet. None of the researchers have any financial disclosures or relation to the Novo Nordisk Foundation. The Novo Nordisk Foundation have had no part in the design of the study, and they will not participate in the reporting of the results. The study participants will not be reimbursed for participation in the study. The trial participants are insured according to the Danish patient compensation scheme.

14. Publication plan

The trial is registered on EudraCT. The trial protocol will be registered at ClinicalTrials.gov before enrollment of the first patient. The protocol will be published prior to unblinding of the results in a methodology article including the statistical analysis plan. The main article will include the primary and secondary outcomes. The tertiary and long-term outcomes will be included in subsequent articles. The manuscripts will be used to report the results of the trial to the scientific community and will adhere to the CONSORT guidelines.[56] The members of the trial coordinating unit will be co-first authors and the coordinating sponsor-investigator will be senior/last author and corresponding author. All site investigators and members of the Trial Steering Committee will be co-authors. Other co-authorships will be decided by contribution according to the ICMJE authorship guidelines[57] depending on personal involvement. All publications will refer to the trial group which will include all contributing parties to the BREAST-AB trial. The manuscripts will be submitted to peer-reviewed international journals and both positive, negative and inconclusive results will be published. The findings of the trial will be shared with participating sites and presented at national and international conferences. The results will be disseminated to the public but will not be shared directly with participating patients. See Gant chart in figure 1.

15. Tasks and Responsibilities

Trial Coordinating Unit: Protocol development, daily management in the trial period, contact to Good Clinical Practice monitoring unit, contact to trial sites, data dictionary development, responsible for providing randomization sequences for each trial site, provide deidentified data to the trial steering committee for safety monitoring, available for unblinding the treatment allocation of individual patients in the case of an emergency, instructing health care personnel involved in the trial, data collection and management.

Coordinating Sponsor-Investigator: Overall responsibility for protocol development, funding, budget overview, ethical approval, trial registration, trial oversight (including enrollment trends and safety), contact to Good Clinical Practice monitoring unit, potential recruitment of additional sites, and dissemination and presentation of results.

Trial Steering Committee: Clinical and scientific advising to the sponsor-investigator, evaluation and recommendations based on yearly reports regarding enrollment trends, progress and safety of the trial, counseling regarding scientific reporting of data, data quality control during long-term follow-up. See 'Trial Steering Committee Charter' for a more thorough description of the tasks and responsibilities of the committee.

Site investigators: Responsible for site-specific enrollment, evaluation and reporting of eligible patients not included, education of personnel at trial sites, reporting of site-specific issues or challenges to the coordinating sponsor-investigator, inclusion of patients, responsible for obtaining informed consent, contact to the regional Good Clinical Practice monitoring unit, trial related data entry in the case report form

Clinical personnel: Preparation and administration of the trial drugs.

Good Clinical Practice-unit: See section 12.

Data Assessment Committee: Assessment and analysis of data.

16. Contact information

Contact for public queries

Mathilde Nejrup Hemmingsen, MD

Department of Plastic Surgery and Burns Treatment

Email: mathilde.nejrup.hemmingsen@regionh.dk

Phone: + 45 35 45 83 69

Contact for scientific queries

Mikkel Herly, MD

Department of Plastic Surgery and Burn Treatments

Blegdamsvej 9, DK2100 Copenhagen

E-mail: mikkel.herly@regionh.dk

Phone: +45 35 45 33 53

17. References

1. Bekæmpelse K. Statestik om brystkræft.
2. 2018 Plastic Surgery Statistics.
3. Coroneos CJ, Selber JC, Offodile AC, Butler CE, Clemens MW. US FDA breast implant postapproval studies: long-term outcomes in 99,993 patients. *Ann Surg*. 2019;269(1):30-36.
4. Stevens WG, Calobrace MB, Harrington J, Alizadeh K, Zeidler KR, d'Incelli RC. Nine-year core study data for Sientra's FDA-approved round and shaped implants with high-strength cohesive silicone gel. *Aesthet Surg J*. 2016;36(4):404-416.
5. Patrick Maxwell G, Van Natta BW, Bengtson BP, Murphy DK. Ten-year results from the natrelle 410 anatomical form-stable silicone breast implant core study. *Aesthetic Surg J*. 2015;35(2):145-155. doi:10.1093/asj/sju084
6. Petersen A, Eftekhari ALB, Damsgaard TE. Immediate breast reconstruction: A retrospective study with emphasis on complications and risk factors. *J Plast Surg Hand Surg*. 2012;46(5):344-348. doi:10.3109/2000656X.2012.700025
7. Dikmans REG, Negenborn VL, Bouman M-B, et al. Two-stage implant-based breast reconstruction compared with immediate one-stage implant-based breast reconstruction augmented with an acellular dermal matrix: an open-label, phase 4, multicentre, randomised, controlled trial. *Lancet Oncol*. 2017;18(2):251-258.
8. Chopra K, Gowda AU, McNichols CHL, Brown EN, Slezak S, Rasko Y. Antimicrobial Prophylaxis Practice Patterns in Breast Augmentation. *Ann Plast Surg*. 2017;78(6):629-632. doi:10.1097/sap.0000000000000942
9. Yalanis GC, Liu E-W, Cheng H-T. Efficacy and safety of povidone-iodine irrigation in reducing the risk of capsular contracture in aesthetic breast augmentation: a systematic review and meta-analysis. *Plast Reconstr Surg*. 2015;136(4):687-698.
10. Kenna DM, Irojah BB, Mudge KL, Eveler K. Absorbable antibiotic beads prophylaxis in immediate breast reconstruction. *Plast Reconstr Surg*. 2018;141(4):486e-492e. doi:10.1097/PRS.0000000000004203
11. Nava MB, Catanuto G, Rocco N. Role of Mitomycin C in Preventing Capsular Contracture in Implant-Based Reconstructive Breast Surgery: A Randomized Controlled Trial. *Plast Reconstr Surg*. 2017;140(6):823e-824e. doi:10.1097/PRS.0000000000003851
12. Hunsicker LM, Chavez-Abraham V, Berry C, McEwen D. Efficacy of vancomycin-based continuous triple antibiotic irrigation in immediate, implant-based breast reconstruction. *Plast Reconstr Surg - Glob Open*. 2017;5(12). doi:10.1097/GOX.0000000000001624
13. Tutela JP, Duncan DP, Kelishadi SS, Chowdhry S, Boyd T, Little JA. Continuous Postoperative Antibiotic Irrigation via Catheter System Following Immediate Breast Reconstruction. *Eplasty*. 2015;15:e49.
14. Hidalgo DA, Sinno S. Current trends and controversies in breast augmentation. *Plast*

Reconstr Surg. 2016;137(4):1142-1150.

15. Lynch JM, Sebai ME, Rodriguez-Unda NA, Seal S, Rosson GD, Manahan MA. Breast pocket irrigation with antibiotic solution at implant insertion: a systematic review and meta-analysis. *Aesthetic Plast Surg*. 2018;42(5):1179-1186.

16. Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med*. 2013;158(3):200-207.

17. Pittet B, Montandon D, Pittet D. Infection in breast implants. *Lancet Infect Dis*. 2005;5(2):94-106. doi:10.1016/S1473-3099(05)01281-8

18. Bartsich S, Ascherman JA, Whittier S, Yao CA, Rohde C. The breast: a clean-contaminated surgical site. *Aesthetic Surg J*. 2011;31(7):802-806. doi:10.1177/1090820X11417428

19. Ahn CY, Ko CY, Wagar EA, Wong RS, Shaw WW. Microbial evaluation: 139 implants removed from symptomatic patients. *Plast Reconstr Surg*. 1996;98(7):1225-1229. doi:10.1097/00006534-199612000-00016

20. Pajkos A, Deva AK, Vickery K, Cope C, Chang L, Cossart YE. Detection of subclinical infection in significant breast implant capsules. *Plast Reconstr Surg*. 2003;111(5):1605-1611.

21. Rieger UM, Mesina J, Kalbermatten DF, et al. Bacterial biofilms and capsular contracture in patients with breast implants. *Br J Surg*. 2013;100(6):768-774.

22. Benediktsson K, Perbeck L. Capsular contracture around saline-filled and textured subcutaneously-placed implants in irradiated and non-irradiated breast cancer patients: Five years of monitoring of a prospective trial. *J Plast Reconstr Aesthetic Surg*. 2006;59(1):27-34. doi:10.1016/j.bjps.2005.08.005

23. Calobrace MB, Stevens WG, Capizzi PJ, Cohen R, Godinez T, Beckstrand M. Risk factor analysis for capsular contracture: A 10-year sientra study using round, smooth, and textured implants for breast augmentation. *Plast Reconstr Surg*. 2018;141(4S):20S-28S. doi:10.1097/PRS.0000000000004351

24. White IV RR, Pitzer KD, Fader RC, Rajab MH, Song J. Pharmacokinetics of topical and intravenous cefazolin in patients with clean surgical wounds. *Plast Reconstr Surg*. 2008;122(6):1773-1779.

25. Berberich C, Sanz-Ruiz P. Risk assessment of antibiotic resistance development by antibiotic-loaded bone cements: Is it a clinical concern? *EFORT Open Rev*. 2019;4(10):576-584. doi:10.1302/2058-5241.4.180104

26. Sweet FA, Roh M, Sliva C. Intrawound application of vancomycin for prophylaxis in instrumented thoracolumbar fusions: efficacy, drug levels, and patient outcomes. *Spine (Phila Pa 1976)*. 2011;36(24):2084-2088.

27. Adams Jr WP, Conner WCH, Barton Jr FE, Rohrich RJ. Optimizing breast-pocket irrigation: the post-betadine era. *Plast Reconstr Surg*. 2001;107(6):1596-1601.

28. Epps MT, Langsdon S, Pels TK, et al. Pocket Irrigation and Technique During Reconstructive Surgery: An American Society of Plastic Surgery Survey of Current Practice. *Ann Plast Surg*.

2019;82(6S Suppl 5):S427-S432. doi:10.1097/SAP.0000000000001790

29. Adams WP, Conner WCH, Barton FE, Rohrich RJ. Optimizing breast pocket irrigation: an in vitro study and clinical implications. *Plast Reconstr Surg*. 2000;105(1):334-338.

30. Darouiche RO, Meade R, Mansouri MD, Netscher DT. In vivo efficacy of antimicrobe-impregnated saline-filled silicone implants. *Plast Reconstr Surg*. 2002;109(4):1352-1357. doi:10.1097/00006534-200204010-00022

31. Zebala LP, Chuntarapas T, Kelly MP, Talcott M, Greco S, Riew KD. Intrawound vancomycin powder eradicates surgical wound contamination: an in vivo rabbit study. *J Bone Joint Surg Am*. 2014;96(1):46-51. doi:10.2106/JBJS.L.01257

32. Yarboro SR, Baum EJ, Dahners LE. Locally administered antibiotics for prophylaxis against surgical wound infection. An in vivo study. *J Bone Joint Surg Am*. 2007;89(5):929-933. doi:10.2106/JBJS.F.00919

33. Cavanaugh DL, Berry J, Yarboro SR, Dahners LE. Better prophylaxis against surgical site infection with local as well as systemic antibiotics. An in vivo study. *J Bone Joint Surg Am*. 2009;91(8):1907-1912. doi:10.2106/JBJS.G.01237

34. Frois AO, Harbour PO, Azimi F, et al. The role of antibiotics in breast pocket irrigation and implant immersion: a systematic review. *Plast Reconstr Surg Glob Open*. 2018;6(9).

35. Drinane JJ, Chowdhry T, Pham T-H, et al. Examining the Role of Antimicrobial Irrigation and Capsular Contracture: A Systematic Review and Meta-analysis. *Ann Plast Surg*. 2017;79(1):107-114. doi:http://dx.doi.org/10.1097/SAP.0000000000001134

36. Campbell CA. The Role of Triple-Antibiotic Saline Irrigation in Breast Implant Surgery. *Ann Plast Surg*. 2018;80(6S Suppl 6):S398-S402. doi:10.1097/SAP.0000000000001345

37. Horsnell JD, Searle AE, Harris PA, et al. Intra-operative techniques to reduce the risk of capsular contracture in patients undergoing aesthetic breast augmentation - A review. *Surgeon*. 2017;15(5):282-289. doi:http://dx.doi.org/10.1016/j.surge.2017.03.001

38. Huang N, Liu M, Yu P, Wu J. Antibiotic prophylaxis in prosthesis-based mammoplasty: a systematic review. *Int J Surg*. 2015;15:31-37.

39. Samargandi OA, Joukhadar N, Al Youha S, Thoma A, Williams J. Antibiotic Irrigation of Pocket for Implant-Based Breast Augmentation to Prevent Capsular Contracture: A Systematic Review. *Plast Surg (Oakville, Ont)*. 2018;26(2):110-119. doi:10.1177/2292550317747854

40. Burkhardt BR, Dempsey PD, Schnur PL, Tofield JJ. Capsular contracture: a prospective study of the effect of local antibacterial agents. *Plast Reconstr Surg*. 1986;77(6):919-932.

41. Stevens WG, Nahabedian MY, Calobrace MB, et al. Risk factor analysis for capsular contracture: a 5-year Sientra study analysis using round, smooth, and textured implants for breast augmentation. *Plast Reconstr Surg*. 2013;132(5):1115-1123.

42. Araco A, Gravante G, Araco F, Delogu D, Cervelli V, Walgenbach K. Infections of breast implants in aesthetic breast augmentations: a single-center review of 3,002 patients.

Aesthetic Plast Surg. 2007;31(4):325-329.

43. Pfeiffer P, Jørgensen S, Kristiansen TB, Jørgensen A, Hölmich LR. Protective effect of topical antibiotics in breast augmentation. *Plast Reconstr Surg*. 2009;124(2):629-634.

44. Giordano S, Peltoniemi H, Lilius P, Salmi A. Povidone-iodine combined with antibiotic topical irrigation to reduce capsular contracture in cosmetic breast augmentation: a comparative study. *Aesthetic Surg J*. 2013;33(5):675-680.

45. Blount AL, Martin MD, Lineberry KD, Kettaneh N, Alfonso DR. Capsular contracture rate in a low-risk population after primary augmentation mammaplasty. *Aesthetic Surg J*. 2013;33(4):516-521.

46. Drinane JJ, Bergman RS, Folkers BL, Kortes MJ. Revisiting triple antibiotic irrigation of breast implant pockets: A placebo-controlled single practice cohort study. *Plast Reconstr Surg - Glob Open*. 2013;1(7). doi:10.1097/GOX.0b013e3182aa8760

47. Drinane JJ, Kortes MJ, Bergman RS, Folkers BL. Evaluation of antibiotic irrigation versus saline irrigation in reducing the long-term incidence and severity of capsular contraction after primary augmentation mammoplasty. *Ann Plast Surg*. 2016;77(1):32-36.

48. Adams WPJ, Rios JL, Smith SJ. Enhancing patient outcomes in aesthetic and reconstructive breast surgery using triple antibiotic breast irrigation: six-year prospective clinical study. *Plast Reconstr Surg*. 2006;117(1):30-36.

49. Gutowski KA, Mesna GT, Cunningham BL. Saline-filled breast implants: A Plastic Surgery Educational Foundation multicenter outcomes study. *Plast Reconstr Surg*. 1997;100(4):1019-1027. doi:10.1097/00006534-199709001-00028

50. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC Definitions of Nosocomial Surgical Site Infections, 1992: A Modification of CDC Definitions of Surgical Wound Infections. *Infect Control Hosp Epidemiol*. 1992;13(10):606-608. doi:10.1086/646436

51. Spear SL, Baker JJL. Classification of capsular contracture after prosthetic breast reconstruction. *Plast Reconstr Surg*. 1995;96(5):1119-1123; discussion 1124.

52. Pusic AL, Klassen AF, Scott AM, Klok JA, Cordeiro PG, Cano SJ. Development of a new patient-reported outcome measure for breast surgery: the BREAST-Q. *Plast Reconstr Surg*. 2009;124(2):345-353.

53. Landspatientsregisteret: Avanceret udtræk.

54. Zalavras CG, Patzakis MJ, Holtom P. Local antibiotic therapy in the treatment of open fractures and osteomyelitis. *Clin Orthop Relat Res*. 2004;427:86-93.

55. Samargandi OA, Joukhdar N, Shaikh OM, Al Youha S, Williams J. Role of Antibiotic Irrigation in Preventing Capsular Contracture and Other Complication After Breast Augmentation: A Systematic Review and Meta-analysis. *Plast Reconstr Surg Glob Open*. 2016;4(9 Suppl).

56. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. *Int J Surg*. 2011;9(8):672-677. doi:10.1016/j.ijsu.2011.09.004

57. International Committee of Medical Journal Editors (ICMJE). International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals: Sample References. 2009;88(May):1-19.

Version 2.8
May 10, 2022

18. Appendices

Appendix 1: Systematic review

Appendix 2: Screening log

Appendix 3: Randomization strata

Appendix 4: Standard operating form for preparation of investigational drugs

Appendix 5: Standard operating form for administration of investigational drugs

Appendix 6: Case report forms

Appendix 7: Medicine account

Appendix 8: Trial Steering Committee Charter

Figure 1: Gantt chart

Figure 2: Participant timeline

Figure 3: Patients

Figure 4: Preparation of treatment

EudraCT: 2020-002459-40
The BREAST-AB Trial
Consent form version 2.0

INVESTIGATORS VERSION: Is to be signed and collected with written consent

Informed consent for participation in a health science research project

Titel: Prophylactic treatment with locally applied antibiotics on breast implants for breast reconstruction

Original titel: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Declaration from the trial participant:

I have received written and oral information about the trial, and I have obtained sufficient knowledge regarding the trial's purpose and methods including any advantages and disadvantages related to participation in the trial.

I am aware that it is voluntary to participate, and that I can withdraw my consent at any time during the trial without losing my current or future rights to treatment.

I hereby give consent to participate in the trial and that those responsible for the trial can obtain information from my medical record. I have received a copy of this consent form along with a copy of the participant information regarding the trial.

Name of the participant: _____

Date: _____ Signature: _____

Do you wish to be informed about the results of the trial?

Yes _____ No _____

Declaration from the investigator providing the oral information:

I hereby declare that the trial participant has received oral and written information regarding the trial.

In my opinion, sufficient information has been provided to enable a decision on participation in trial.

The name of the investigator who provided the information:

Date: _____ Signature: _____

This study is approved by the Danish Data Protection Agency (P-2019-219), The Ethics Committee of the Capital Region (H-20046592) and the Danish Medicines Agency (2020070016)

EudraCT: 2020-002459-40
The BREAST-AB Trial
Consent form version 2.0

PARTICIPANTS VERSION: handed over to the participant

Informed consent for participation in a health science research project

Titel: Prophylactic treatment with locally applied antibiotics on breast implants for breast reconstruction

Original titel: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Declaration from the trial participant:

I have received written and oral information about the trial, and I have obtained sufficient knowledge regarding the trial's purpose and methods including any advantages and disadvantages related to participation in the trial.

I am aware that it is voluntary to participate, and that I can withdraw my consent at any time during the trial without losing my current or future rights to treatment.

I hereby give consent to participate in the trial and that those responsible for the trial can obtain information from my medical record. I have received a copy of this consent form along with a copy of the participant information regarding the trial.

Name of the participant: _____

Date: _____ Signature: _____

Do you wish to be informed about the results of the trial?

Yes _____ No _____

Declaration from the investigator providing the oral information:

I hereby declare that the trial participant has received oral and written information regarding the trial.

In my opinion, sufficient information has been provided to enable a decision on participation in trial.

The name of the investigator who provided the information:

Date: _____ Signature: _____

This study is approved by the Danish Data Protection Agency (P-2019-219), The Ethics Committee of the Capital Region (H-20046592) and the Danish Medicines Agency (2020070016)

EudraCT: 2020-002459-40
The BREAST-AB Trial
Samtykkeerklæring version 2.0

FORSKERS VERSION: underskrives og indhentes med skriftligt samtykke

Informeret samtykke til deltagelse i et sundhedsvidenskabeligt forskningsprojekt.

Titel: Forebyggende behandling med lokal antibiotika af brystimplantater til brystrekonstruktion

Originaltitel: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Erklæring fra forsøgspersonen:

Jeg har fået skriftlig og mundtlig information, og jeg ved nok om studiets formål, metode, fordele og ulemper til at sige ja til at deltage.

Jeg ved, at det er frivilligt at deltage, og at jeg altid kan trække mit samtykke tilbage uden at miste mine nuværende eller fremtidige rettigheder til behandling.

Jeg giver samtykke til at deltage i forskningsprojektet, samt til at projektansvarlige kan tilgå mine journaloplysninger. Jeg har fået en kopi af dette samtykkeark samt en kopi af den skriftlige information om projektet til eget brug.

Forsøgspersonens navn:_____

Dato: _____Underskrift: _____

Ønsker du at blive informeret om forskningsprojektets resultat?

Ja _____ (sæt x) Nej _____ (sæt x)

Erklæring fra den, der afgiver information:

Jeg erklærer, at forsøgspersonen har modtaget mundtlig og skriftlig information om forsøget.

Efter min overbevisning er der givet tilstrækkelig information, til at der kan træffes beslutning om deltagelse i forsøget.

Navnet på den, der har afgivet information:

Dato: _____Underskrift: _____

Dette studie er godkendt af Region Hovedstadens datatilsyn (P-2019-219), Region Hovedstadens Videnskabsetiske Komite (H-20046592) og Lægemiddelstyrelsen (2020070016)

EudraCT: 2020-002459-40
The BREAST-AB Trial
Samtykkeerklæring version 2.0

FORSØGSDELTAGERS VERSION: rives af og gives til forsøgsdeltager

Informeret samtykke til deltagelse i et sundhedsvidenskabeligt forskningsprojekt.

Titel: Forebyggende behandling med lokal antibiotika af brystimplantater til brystrekonstruktion

Originaltitel: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Erklæring fra forsøgspersonen:

Jeg har fået skriftlig og mundtlig information, og jeg ved nok om studiets formål, metode, fordele og ulemper til at sige ja til at deltage.

Jeg ved, at det er frivilligt at deltage, og at jeg altid kan trække mit samtykke tilbage uden at miste mine nuværende eller fremtidige rettigheder til behandling.

Jeg giver samtykke til at deltage i forskningsprojektet, samt til at projektansvarlige kan tilgå mine journaloplysninger. Jeg har fået en kopi af dette samtykkeark samt en kopi af den skriftlige information om projektet til eget brug.

Forsøgspersonens navn:_____

Dato: _____Underskrift: _____

Ønsker du at blive informeret om forskningsprojektets resultat?

Ja _____ (sæt x) Nej _____ (sæt x)

Erklæring fra den, der afgiver information:

Jeg erklærer, at forsøgspersonen har modtaget mundtlig og skriftlig information om forsøget.

Efter min overbevisning er der givet tilstrækkelig information, til at der kan træffes beslutning om deltagelse i forsøget.

Navnet på den, der har afgivet information:

Dato: _____Underskrift: _____

Dette studie er godkendt af Region Hovedstadens datatilsyn (P-2019-219), Region Hovedstadens Videnskabsetiske Komite (H-20046592) og Lægemiddelstyrelsen (2020070016)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	2
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
	2b	All items from the World Health Organization Trial Registration Data Set	4-18
Protocol version	3	Date and version identifier	Full protocol in supplementary material (SM)
Funding	4	Sources and types of financial, material, and other support	18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	3, 18
	5b	Name and contact information for the trial sponsor	3, 18
	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	18
	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)	18
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	6

	6b	Explanation for choice of comparators	Full protocol in SM
Objectives	7	Specific objectives or hypotheses	6, 9-10
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, noninferiority, exploratory)	7-8, 10-11
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7, full protocol in SM
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)	7-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	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)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7-8
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	9-11
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)	See Figure 2
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	11

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	NA
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	8-9
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	Full protocol in SM
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Full protocol in SM
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	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. Reference to where data collection forms can be found, if not in the protocol	9-11, full protocol in SM
	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	Full protocol in SM
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	Full protocol in SM

Statistical methods	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	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	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)	Full protocol in SM
Methods: Monitoring			
Data monitoring	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	11, Full protocol in SM
	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	Full protocol in SM
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12, Full protocol in SM
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Full protocol in SM
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4, 12
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)	4, full protocol in SM
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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	Full protocol in SM

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
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	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	Full protocol in SM
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Example of patient consent form in SM
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	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: Protocol for a randomized, double-blind, placebo-controlled trial (The BREAST-AB trial)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-058697.R2
Article Type:	Protocol
Date Submitted by the Author:	16-Aug-2022

Complete List of Authors:	Hemmingsen, Mathilde; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Larsen, Andreas; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Weltz, Tim; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Ørholt, Mathias; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Wiberg, Sebastian; Zealand University Hospital Koge, Department of Anaesthesiology Bennedsen, Anne; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Bille, Camilla; Odense University Hospital, Department of Plastic Reconstructive Surgery Carstensen, Lena; Southwest Jutland Hospital, Department of Plastic Surgery Jensen, Lisa; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Bredgaard, Rikke; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Herlev og Gentofte Koudahl, Vibeke; Lillebaelt Hospital, Department of Plastic Surgery Schmidt, Volker ; Zealand University Hospital Roskilde, Department of Plastic and Breast Surgery; University of Copenhagen, Department of Clinical Medicine Vester-Glowinski, Peter; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet Hölmich, Lisbet; Copenhagen University Hospital, Department of Plastic and Reconstructive Surgery, Herlev og Gentofte; University of Copenhagen, Department of Clinical Medicine Sørensen, Søren; University of Copenhagen, Department of Biology, Section of Microbiology Bjarnsholt, Thomas; University of Copenhagen, Department of Immunology and Microbiology; Copenhagen University Hospital, Department of Clinical Microbiology, Rigshospitalet Damsgaard, Tine; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet; University of Copenhagen, Department of Clinical Medicine

	Herly, Mikkel; Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet; University of Copenhagen, Department of Immunology and Microbiology
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Evidence based practice
Keywords:	Plastic & reconstructive surgery < SURGERY, Breast surgery < SURGERY, Clinical trials < THERAPEUTICS

Title: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: Protocol for a randomized, double-blind, placebo-controlled trial (The BREAST-AB trial)

Authors

Mathilde Nejrup Hemmingsen, MD[1]; Andreas Larsen, MD[1]; Tim Kongsmark Weltz, MD[1]; Mathias Ørholt, MD[1]; Sebastian Wiberg, MD[2]; Anne Karen Bennedsen, BMSc[1]; Camilla Bille, MD[3]; Lena Felicia Carstensen, MD[4]; Lisa Toft Jensen, MD[1]; Rikke Bredgaard, MD[5]; Vibeke Koudahl, MD[6]; Volker Jürgen Schmidt, MD[7,8]; Peter Viktor Vester-Glowinski, MD[1]; Lisbet Rosenkrantz Hölmich[5,8], MD; Søren J Sørensen, MSc[9]; Thomas Bjarnsholt, MSc[10,11]; Tine Damsgaard, MD[1,8]; Mikkel Herly, MD[1,10]

Affiliations

[1]Copenhagen University Hospital, Department of Plastic Surgery and Burns Treatment, Rigshospitalet, Copenhagen, Denmark

[2]Zealand University Hospital Køge, Department of Anaesthesiology, Køge, Denmark

[3]Odense University Hospital, Department of Plastic Reconstructive Surgery, Odense, Denmark

[4]Southwest Jutland Hospital, Department of Plastic Surgery, Esbjerg, Denmark

[5]Copenhagen University Hospital, Department of Plastic and Reconstructive Surgery, Herlev and Gentofte, Herlev, Denmark

[6]Lillebaelt Hospital, Department of Plastic Surgery, Vejle, Denmark

[7]Zealand University Hospital Roskilde, Department of Plastic and Breast Surgery, Roskilde, Denmark

[8]University of Copenhagen, Department of Clinical Medicine, Copenhagen, Denmark

[9]University of Copenhagen, Department of Biology, Section of Microbiology, Copenhagen, Denmark

[10]University of Copenhagen, Department of Immunology and Microbiology, Copenhagen, Denmark

[11]Copenhagen University Hospital, Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

Corresponding author

Mathilde Nejrup Hemmingsen, MD

Department of Plastic Surgery and Burns Treatment

Copenhagen University Hospital, Rigshospitalet

Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Email: mathilde.nejrup.hemmingsen@regionh.dk

Phone: +45 285 682 05

Keywords: Breast reconstruction, implant irrigation, infection, capsular contracture

Word count: 2.914

Abstract (257/300)

Introduction

Periprosthetic infection is one of the most severe complications following implant-based breast reconstruction affecting 5-10% of the women. Currently, many surgeons apply antibiotics locally on the breast implant to reduce the risk of postoperative infection, but no randomized, placebo-controlled trials have tested the treatment's efficacy.

Methods and analysis

The BREAST-AB trial is an investigator-initiated, multicentre, randomized, placebo-controlled, double-blind trial of local treatment with gentamicin, vancomycin and cefazolin on breast implants in women undergoing implant-based breast reconstruction. The trial drug consists of 80 mg gentamicin, 1 g vancomycin and 1 g cefazolin dissolved in 500 ml of isotonic saline. The placebo solution consists of 500 ml isotonic saline. The trial drug is used to wash the dissected tissue pocket and the breast implant prior to insertion. The primary outcome is all-cause explantation of the breast implant within 180-days after the breast reconstruction surgery. This excludes cases where the implant is replaced with a new permanent implant e.g., for cosmetic reasons. Key long-term outcomes include capsular contracture and quality of life. The trial started on 26 January 2021 and is currently recruiting.

Ethics and dissemination

The trial was approved by the Regional Ethics Committee of the Capital Region (H-20056592) on 1 January 2021 and the Danish Medicines Agency (2020070016) on 2 August 2020. The main paper will include the primary and secondary outcomes and will be submitted to an international peer-reviewed journal.

Registration

The trial was registered at ClinicalTrials.gov (NCT 04731025) on 29 January 2021 and at the EU Clinical Trials Register (EudraCT 2020-002459-40) on 17 December 2020.

Strengths and limitations of this study

- The trial will include all types of patients undergoing breast reconstruction surgery with implants which makes the results relevant for all women undergoing implant-based breast reconstruction
- The randomized design will ensure an even distribution of risk factors in the placebo- and intervention group which will isolate the intervention's effect on the outcome
- The women undergoing bilateral breast reconstruction will receive antibiotic treatment to one of their breasts and placebo to the contralateral breast which isolate the effect of the trial drug from inter-individual variation
- The trial is evaluated with endpoints that are of great importance for patients
- The incidence of the primary outcome is relatively low, so despite the large sample size, a small effect of the treatment may not be detected with statistical significance

Text (2.914/4.000 words)

Introduction

Breast reconstruction has been shown to improve a woman's quality of life after undergoing breast cancer surgery.[1] An increasing number of women choose implant-based breast reconstruction[2] which includes a risk of implant infection. Implant infection is seen in 5-10% of the women,[3]–[7] and the treatment typically requires removal of the implant after which the patient must wait several months before a new breast reconstruction can be attempted.

Previous studies suggest that bacterial contamination of the breast implant can occur without any clinical symptoms.[8], [9] Instead, the bacteria form a chronic, subclinical infection which is suspected to cause a prolonged immune reaction to the implant called capsular contracture which affects up to 10-20% of the patients.[10], [11] Capsular contracture causes hardening and deformity of the breast, and the treatment often includes surgical removal of the contracted capsule and exchange of the implant.

Surgeons have attempted numerous strategies to prevent bacterial contamination of the implant.[12]–[17] The most widely followed approach is to apply antibiotics directly on the breast implant and in the dissected tissue pocket during the surgery,[18] but only few studies have investigated the clinical effect of the treatment. A recent meta-analysis found a decreased rate of implant infection and capsular contracture in women treated with antibiotics applied on the breast implant.[19] However, the included studies were mostly retrospective and varied greatly in the applied antibiotics, control groups and follow-up period. Furthermore, no randomized controlled trials were identified in the meta-analysis.[19]

Due to the limited evidence, The Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection has no recommendations regarding the use of locally applied antibiotics on implants[20] and The National Institute for Health and Care Excellence (NICE) in England has requested further studies investigating the clinical effect of the treatment.[21] Randomized clinical trials are essential for developing evidence-based treatment guidelines. The BREAST-AB trial is designed to assess the effect of locally applied antibiotics on all-cause loss of the implant after implant-based breast reconstruction. We hypothesize that local application of gentamicin, vancomycin and cefazolin decrease the risk of postoperative clinical infections and

thereby reduce the risk of losing the implant to the benefit of women undergoing implant-based breast reconstruction.

Methods and analysis

The protocol was written in accordance with the SPIRIT statement[22] and the ICH-GCP guidelines.[23] The protocol is provided in full length in supplementary file 1.

Trial design

The BREAST-AB trial is an investigator-initiated, multicentre, randomized, double-blind, placebo-controlled trial investigating local application of gentamicin, vancomycin and cefazolin during implant-based breast reconstruction. The antibiotic solution or placebo is applied directly onto the breast implant and in the dissected tissue pocket during the surgery.

Setting

The trial will be conducted at six hospitals in Denmark, and additional trial sites may be included during the trial period. See supplementary file 1 for a list of the trial sites.

Eligibility criteria

The trial will include all types of patients undergoing breast reconstruction surgery with implants which makes the results relevant for all women undergoing implant-based breast reconstruction. Patients that meet the following criteria are considered eligible for inclusion:

- Age ≥ 18
- Biologically female
- Written informed consent
- Scheduled for breast reconstruction with implants or expanders including
  - immediate or delayed reconstruction
  - unilateral or bilateral reconstruction
  - with or without simultaneous flap reconstruction

Exclusion criteria are:

- Pregnancy

- Breast feeding
- Known allergy towards gentamicin, vancomycin, cefazolin or neomycin
- Known anaphylactic reaction towards beta-lactam antibiotics or aminoglycosides
- Myasthenia gravis
- Known impaired renal function, GFR < 60 ml/min
- Participation in investigational drug trials concerning disinfection agents in the breast cavity

Trial intervention

The trial drug contains 80 mg gentamicin, 1000 mg vancomycin and 1000 mg cefazolin dissolved in an infusion bag containing 500 ml of sterile isotonic saline. The placebo solution consists of 500 ml sterile isotonic saline contained in a similar infusion bag. Both solutions are achromatic, and the infusion bags are indistinguishable from one another. See figure 1 for an illustration of the trial intervention.

During the surgery, the responsible nurse draws 150 ml from the assigned infusion bag and the plastic surgeon uses it to wash the dissected tissue pocket.[24] Another 50 ml are drawn from the same infusion bag and used to soak the implant prior to insertion in the tissue pocket. The rest of the content in the infusion bag is discarded.

Randomization

The trial drug and placebo are assigned in a 1:1 ratio. Patients undergoing unilateral breast reconstruction are randomized to either the trial drug or placebo, whereas patients who undergo bilateral breast reconstruction are randomized to the trial drug on one breast and placebo on the contralateral breast. The paired design involving the patients undergoing bilateral surgery isolates the effect of the trial treatment from the inter-individual variation, as these patients serve as their own control. Patients who undergo two-stage breast reconstruction with an expander implant that is replaced with a permanent implant after three to six months are allocated to the same trial treatment during both surgeries. See figure 2 for an overview of the trial design.

The randomization is stratified according to study site; whether the patients undergo unilateral- or bilateral surgery; and selected risk factors based on the literature[25] including radiation therapy

and immediate- versus delayed reconstruction. This approach ensures an even distribution of the selected risk factors in the placebo group and the intervention group. The randomized design will ensure that other potential risk factors, which are not included in the stratification, are evenly distributed in the intervention- and control group. The treatment is assigned in a fixed block size of two to ensure that the trial drug and placebo is evenly distributed within each stratum.

Blinding

The trial is double-blind so that the patients, site investigators, health care personnel and the data assessors are blinded to the allocated treatment. The only unblind investigators are the nurses responsible for preparing the trial drugs and the members of the trial coordination unit who provide the treatment allocation. The designated nurse prepares the trial drugs before the surgery. The trial drugs are prepared outside of the operating room to make sure that the surgeon and the surgical staff are blinded to the treatment. The unblind investigators do not take part in any treatment-related procedures, clinical evaluation of the outcomes or data assessment. In case of emergency unblinding, the trial coordination unit will provide the allocation assignment under discretion of the treating physician.

Primary outcome

All-cause explantation of the breast implant within 180-days after the breast reconstruction

All-cause explantation is defined as explantation and discarding of the breast implant. However, the following cases are not counted as explantation: replacement of an expander with a permanent implant; and replacement of a permanent breast implant with a new permanent breast implant due to cosmetic revisions such as asymmetry, implant malposition, change of size or implant rotation.

The rationale for the primary outcome is to quantify whether the locally applied antibiotics prevent severe infection or other complications that leads to loss of the reconstruction within 180 days after surgery. Sometimes, the indication for explantation of the implant may be ambiguous because multiple complications can occur simultaneously. Therefore, all-cause explantation was chosen as a more objective alternative to infection that leads to explantation of the implant. The primary outcome does not include explantation and direct placement of a new permanent implant

for cosmetic reasons because revisional surgery is not considered a proxy for severe complications that may be affected by antibiotics on the implant.

The definition of explantation for cosmetic reasons and discarding of the breast implant was revised in the protocol V2.8, dated 10 May 2022, from only mentioning asymmetry and implant rotation to include all types of implant malposition and change of implant size.

Secondary outcomes

Time to explantation

Time to explantation is defined as the number of days from the reconstructive surgery to the surgical removal of the implant. This outcome was chosen because local application of antibiotics may delay the development of a postoperative clinical infection.

All-cause explantation of the breast implant within 1 year after the breast reconstruction surgery (Y/N)

Previous studies[26] suggest that surgical removal of the permanent implant can occur up to 1 year after the surgery, and therefore this is included as a secondary outcome.

Revision surgery with incision of the fibrous capsule after the breast reconstruction surgery

This is defined as all revisional surgery that includes exposure of the breast implant. This outcome was included because the breast reconstruction in some cases can be upheld with revisional surgery despite complications that may be associated with low-virulent bacteria.

Exchange of the permanent implant with an expander implant after the breast reconstructive surgery

This subgroup consists of patients who undergo a salvage procedure, where the permanent implant is removed and discarded, and the implant pocket is cleansed and irrigated with antiseptic agents (e.g., a solution of hydrogen peroxide) after which an expander implant is inserted to prevent the tissue envelope from contracting while still preserving the vulnerable skin flaps. This group will be counted in the primary outcome, but we hypothesize that the patients chosen for this treatment option may have less severe symptoms of infection and may have concomitant necrosis due to poor blood supply to the skin flaps. Therefore, the effect of the treatment in this

subgroup may be modest compared to the patients who have explantation without replacing it with an expander.

Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction

Surgical site infection is defined according to the CDC classification.[27] The clinical signs of infection are combined with the prescription of antibiotics as a confirmation of the surgeons suspicion of infection. Additional outcome measures are listed in supplementary file 1 and on ClinicalTrials.gov.

Long-term outcomes

The trial includes a long-term assessment of capsular contracture after 5, 10 and 15 years. The use of locally applied antibiotics could potentially decrease the rate of capsular contracture by minimizing or altering the low-virulent bacterial contamination of the implant. Therefore, capsular contracture is an important long-term outcome.

The trial also evaluates long-term quality of life using the BREAST-Q questionnaire 'Reconstruction Module'.[28] The preoperative questionnaire is administered after the patient has provided informed consent. The postoperative questionnaire is administered 3 months, 1 year and 5 years postoperatively. The application of local antibiotics may decrease the risk of postoperative complications and thereby decrease the risk of undergoing revision surgery. This in turn may lead to improved patient satisfaction and quality of life. BREAST-Q is a validated tool used to quantify patient satisfaction and health-related quality of life after breast reconstruction surgery.

Sample size

The trial is powered to find a 5% risk reduction in the primary outcome. Based on the literature, the assumed rate of implant loss in the control group is 10%.[6], [7] The independent sample unit is "breast", because previous data do not suggest that implant loss is correlated between the two breasts of a patient.[3] Therefore, the power of the trial is based on the number of breasts, so that the final number of included patients depends on the proportion of patients who undergo bilateral breast reconstruction. With an alpha of 0.05, the trial will have a power of 0.90 to detect an absolute risk reduction of 5% with 1158 breasts. To account for drop-out of up to 10%, we will

include patients with a combined estimated number of 1274 breasts in the trial. We expect 27% of the patients to undergo bilateral breast reconstruction (based on unpublished data) and therefore, 1003 patients will be included in the trial.

Statistical analysis plan

The statistical analyses and -reporting will adhere to the CONSORT guidelines.[29] All statistical analyses will be conducted on a modified intention-to-treat population defined as all patients that have been allocated to the study drug and have a valid informed consent.

The primary outcome and key secondary outcomes are categorical variables and will be presented as frequencies in each group. The overall effect of the intervention on the primary and secondary outcomes will be modelled as both univariate and multivariate mixed effects logistic regression models considering the correlation between breasts in patients undergoing bilateral surgery. The results will be presented as crude and adjusted odds ratios (OR) with 95% confidence intervals. The model will be adjusted for potential confounders, including age, smoking, body mass index, trial site and indication for surgery (prophylactic mastectomy versus mastectomy after cancer diagnosis). The full statistical analysis plan is provided in the protocol in supplementary file 1.

Data collection and follow-up

All patients are admitted to the hospital for approximately 3 days after the surgery. All patients are scheduled for postoperative follow-up visits after approximately 3 months and 1 year. Data on drug administration are obtained real-time and entered in an electronic case report form. Additional data are obtained from the patients' medical records by trained researchers and entered in the electronic case report form. A list of included variables is provided in the protocol in supplementary file 1.

Clinical treatment

Participation in the trial will not interfere with any clinical decisions regarding the treatment of the patients, and all other clinical treatment than the trial treatment, including pre-, peri- and postoperatively administered medicine, will adhere to the standard treatment at each trial site. The randomized design and the stratified randomization will ensure that potential risk factors which may influence the outcome are equally distributed in the placebo- and intervention group.

Patient and public involvement

None.

Ethics and dissemination

Ethical considerations

The trial protocol has been reviewed and approved by the Regional Ethics Committee of the Capital Region (H-20056592) on 1 January 2021 and the Danish Medicines Agency (EudraCT 2020-002459-40) on 2 August 2020. The trial is monitored by the Good Clinical Practice units in Denmark.

There are currently no clinical guidelines in Denmark regarding the use of locally applied antibiotics on breast implants, and the treatment depends on the individual surgeon's preference. Allocation to placebo in this trial is therefore considered ethically acceptable. A detailed description of the ethical considerations is provided in the supplementary file 1.

Safety considerations

Previous studies have shown that the serum level after local application of antibiotics is low,[30], [31] and therefore the risk of systemic side effects is low. Gentamicin, vancomycin and cefazolin have been used for local application on breast implants for many years and are considered safe.[16], [32] If the patients experience adverse events, it will be registered in an electronic case report form in REDCap and treated according to local guidelines. All serious adverse reactions will be reported yearly to the Danish Medicines Agency and the Regional Ethics Committee by the sponsor-investigator during the study period. A more detailed description of the safety considerations is provided in the protocol in supplementary file 1.

Consent

Consent from trial participants is obtained according to Danish legislation.[33] The investigators are responsible for obtaining the signed, informed consent from the patients prior to any protocol-related activities. The consent can be withdrawn by the patient at any time and without explanation, after which the patient will receive the standard treatment according to the local guidelines. An example of the patient consent form is provided in supplementary file 2 and 3.

Dissemination

The main paper will include the primary and secondary outcomes. The manuscript will adhere to the CONSORT guidelines and will be used to report the results of the trial to the scientific community. The manuscript will be submitted to an international peer-reviewed journal, and both positive, negative and inconclusive results will be published. The findings of the trial will be shared with participating sites and presented at national and international conferences. The results will be registered at ClinicalTrials.gov and will be disseminated to the public.

Status

The first patient was enrolled in the trial in January 2021, and the trial is currently recruiting. The last patient is expected to be included in January 2025. The primary results of the trial are anticipated in July 2025 after the last patient's last follow-up. The results from this trial can be used in evidence-based treatment guidelines for implant-based breast reconstruction surgery.

Author contributions

All authors took part in designing the trial and writing the trial protocol. Mathilde Nejrup Hemmingsen, Andreas Larsen, Tim K Weltz and Anne K Bennedsen constitutes the trial coordinating unit who are responsible for coordinating all trial related activities. Mathilde Nejrup Hemmingsen has written the manuscript. Mikkel Herly is the Coordinating Principal Investigator of the trial and the local site investigator at Rigshospitalet. He has critically revised and contributed to the final version of the manuscript. Tine Damsgaard, Søren J Sørensen, Thomas Bjarnsholt, Peter Vester-Glowinski and Mikkel Herly are members of the trial steering committee and they have all revised and approved the final manuscript. Camilla Bille, Rikke Bredgaard, Lena F Carstensen, Lisbet Rosenkrantz Hölmich, Lisa Toft Jensen, Vibeke Koudahl and Volker J Schmidt are local site investigators and they have revised and approved the final manuscript. Mathias Ørholt and Sebastian Wiberg are members of the blinded data assessment committee and have provided statistical expertise to the trial design and contributed with writing the statistics sections and have revised and approved the final manuscript.

Competing interests

None declared.

Funding and sponsor

Mikkel Herly is the initiator and sponsor of the trial. The trial is supported by the Novo Nordisk Foundation (grant number 0058322, grant holder Tine Damsgaard) and the Medicine Fund of the Danish Regions (grant number R-189-A4127, grant holder Mikkel Herly).

Data sharing statement

After publication of the results, researchers and other relevant parties can be granted access to anonymized data upon request.

References

[1] Y. Eltahir *et al.*, "Quality-of-life outcomes between mastectomy alone and breast reconstruction: comparison of patient-reported BREAST-Q and other health-related quality-of-life measures," *Plast. Reconstr. Surg.*, vol. 132, no. 2, pp. 201e-209e, 2013.

[2] American Society of Plastic Surgeons, "2019 Plastic Surgery Statistics Report," 2019. [Online]. Available: https://www.plasticsurgery.org/documents/News/Statistics/2019/plastic-surgery-statistics-full-report-2019.pdf.

[3] C. J. Coroneos, J. C. Selber, A. C. Offodile, C. E. Butler, and M. W. Clemens, "US FDA breast implant postapproval studies: long-term outcomes in 99,993 patients," *Ann. Surg.*, vol. 269, no. 1, pp. 30–36, 2019.

[4] W. G. Stevens, M. B. Calobrace, K. Alizadeh, K. R. Zeidler, J. L. Harrington, and R. C. d'Incelli, "Ten-year Core Study Data for Sientra's Food and Drug Administration–Approved Round and Shaped Breast Implants with Cohesive Silicone Gel," *Plast. Reconstr. Surg.*, vol. 141, no. 4S, pp. 7S-19S, 2018.

[5] G. P. Maxwell, B. W. Van Natta, B. P. Bengtson, and D. K. Murphy, "Ten-year results from the Natrelle 410 anatomical form-stable silicone breast implant core study," *Aesthetic Surg. J.*, vol. 35, no. 2, pp. 145–155, 2015.

[6] A. Petersen, A. L. B. Eftekhari, and T. E. Damsgaard, "Immediate breast reconstruction: a retrospective study with emphasis on complications and risk factors," *J. Plast. Surg. Hand Surg.*, vol. 46, no. 5, pp. 344–348, 2012.

[7] R. E. G. Dikmans *et al.*, "Two-stage implant-based breast reconstruction compared with immediate one-stage implant-based breast reconstruction augmented with an acellular dermal matrix: an open-label, phase 4, multicentre, randomised, controlled trial," *Lancet Oncol.*, vol. 18, no. 2, pp. 251–258, 2017.

[8] A. Pajkos, A. K. Deva, K. Vickery, C. Cope, L. Chang, and Y. E. Cossart, "Detection of subclinical infection in significant breast implant capsules," *Plast. Reconstr. Surg.*, vol. 111,

no. 5, pp. 1605–1611, 2003.

[9] U. M. Rieger *et al.*, "Bacterial biofilms and capsular contracture in patients with breast implants," *Br. J. Surg.*, vol. 100, no. 6, pp. 768–774, 2013.

[10] K. Benediktsson and L. Perbeck, "Capsular contracture around saline-filled and textured subcutaneously-placed implants in irradiated and non-irradiated breast cancer patients: Five years of monitoring of a prospective trial," *J. Plast. Reconstr. Aesthetic Surg.*, vol. 59, no. 1, pp. 27–34, 2006, doi: 10.1016/j.bjps.2005.08.005.

[11] M. B. Calobrace, W. G. Stevens, P. J. Capizzi, R. Cohen, T. Godinez, and M. Beckstrand, "Risk factor analysis for capsular contracture: A 10-year sientra study using round, smooth, and textured implants for breast augmentation," *Plast. Reconstr. Surg.*, vol. 141, no. 4S, pp. 20S-28S, 2018, doi: 10.1097/PRS.0000000000004351.

[12] K. Chopra, A. U. Gowda, C. H. L. McNichols, E. N. Brown, S. Slezak, and Y. Rasko, "Antimicrobial Prophylaxis Practice Patterns in Breast Augmentation: A National Survey of Current Practice.," *Ann. Plast. Surg.*, vol. 78, no. 6, pp. 629–632, Jun. 2017, doi: 10.1097/SAP.0000000000000942.

[13] G. C. Yalanis, E.-W. Liu, and H.-T. Cheng, "Efficacy and safety of povidone-iodine irrigation in reducing the risk of capsular contracture in aesthetic breast augmentation: a systematic review and meta-analysis," *Plast. Reconstr. Surg.*, vol. 136, no. 4, pp. 687–698, 2015.

[14] D. M. Kenna, B. B. Irojah, K. L. Mudge, and K. Eveler, "Absorbable antibiotic beads prophylaxis in immediate breast reconstruction," *Plast. Reconstr. Surg.*, vol. 141, no. 4, pp. 486e-492e, 2018, doi: 10.1097/PRS.0000000000004203.

[15] M. B. Nava, G. Catanuto, and N. Rocco, "Reply: Role of Mitomycin C in Preventing Capsular Contracture in Implant-Based Reconstructive Breast Surgery: A Randomized Controlled Trial," *Plast. Reconstr. Surg.*, vol. 140, no. 6, pp. 823e-824e, 2017, doi: 10.1097/PRS.0000000000003851.

[16] L. M. Hunsicker, V. Chavez-Abraham, C. Berry, and D. McEwen, "Efficacy of vancomycin-based continuous triple antibiotic irrigation in immediate, implant-based breast

reconstruction," *Plast. Reconstr. Surg. - Glob. Open*, vol. 5, no. 12, 2017, doi: 10.1097/GOX.0000000000001624.

[17] J. P. Tutela, D. P. Duncan, S. S. Kelishadi, S. Chowdhry, T. Boyd, and J. A. Little, "Continuous Postoperative Antibiotic Irrigation via Catheter System Following Immediate Breast Reconstruction.," *Eplasty*, vol. 15, p. e49, 2015.

[18] D. A. Hidalgo and S. Sinno, "Current trends and controversies in breast augmentation," *Plast. Reconstr. Surg.*, vol. 137, no. 4, pp. 1142–1150, 2016.

[19] J. M. Lynch, M. E. Sebai, N. A. Rodriguez-Unda, S. Seal, G. D. Rosson, and M. A. Manahan, "Breast pocket irrigation with antibiotic solution at implant insertion: a systematic review and meta-analysis," *Aesthetic Plast. Surg.*, vol. 42, no. 5, pp. 1179–1186, 2018.

[20] S. I. Berriós-Torres *et al.*, "Centers for disease control and prevention guideline for the prevention of surgical site infection, 2017," *JAMA Surg.*, vol. 152, no. 8, pp. 784–791, 2017, doi: 10.1001/jamasurg.2017.0904.

[21] National Institute for Health and Care Excellence (NICE), "Surgical site infections: prevention and treatment." https://www.nice.org.uk/guidance/ng125/chapter/Recommendations#intraoperative-phase.

[22] A.-W. Chan *et al.*, "SPIRIT 2013 statement: defining standard protocol items for clinical trials," *Ann. Intern. Med.*, vol. 158, no. 3, pp. 200–207, 2013.

[23] I. C. H. H. T. Guideline, "General considerations for clinical trials E8," in *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use*, 1997.

[24] W. P. Adams Jr, J. L. Rios, and S. J. Smith, "Enhancing patient outcomes in aesthetic and reconstructive breast surgery using triple antibiotic breast irrigation: six-year prospective clinical study," *Plast. Reconstr. Surg.*, vol. 117, no. 1, pp. 30–36, 2006.

[25] M. A. Olsen, K. B. Nickel, I. K. Fox, J. A. Margenthaler, A. E. Wallace, and V. J. Fraser, "Comparison of Wound Complications After Immediate, Delayed, and Secondary Breast

Reconstruction Procedures.," *JAMA Surg.*, vol. 152, no. 9, p. e172338, Sep. 2017, doi: 10.1001/jamasurg.2017.2338.

[26] J. Kalstrup, C. B. Willert, M. B.-M. Weitemeyer, A. H. Chakera, and L. R. Hölmich, "Immediate direct-to-implant breast reconstruction with acellular dermal matrix: Evaluation of complications and safety," *The Breast*, vol. 60, pp. 192–198, 2021.

[27] H. TC, "Gaynes RP. Martone WJ. Jarvis WR. Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections," *Infect Control Hosp Epidemiol*, vol. 13, pp. 606–608, 1992.

[28] A. L. Pusic, A. F. Klassen, A. M. Scott, J. A. Klok, P. G. Cordeiro, and S. J. Cano, "Development of a new patient-reported outcome measure for breast surgery: the BREAST-Q," *Plast. Reconstr. Surg.*, vol. 124, no. 2, pp. 345–353, 2009.

[29] K. F. Schulz, D. G. Altman, D. Moher, and C. Group, "CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials," *Trials*, vol. 11, no. 1, p. 32, 2010.

[30] F. A. Sweet, M. Roh, and C. Sliva, "Intrawound application of vancomycin for prophylaxis in instrumented thoracolumbar fusions: efficacy, drug levels, and patient outcomes," *Spine (Phila. Pa. 1976).*, vol. 36, no. 24, pp. 2084–2088, 2011.

[31] R. R. White IV, K. D. Pitzer, R. C. Fader, M. H. Rajab, and J. Song, "Pharmacokinetics of topical and intravenous cefazolin in patients with clean surgical wounds," *Plast. Reconstr. Surg.*, vol. 122, no. 6, pp. 1773–1779, 2008.

[32] C. A. Campbell, "The Role of Triple-Antibiotic Saline Irrigation in Breast Implant Surgery," *Ann. Plast. Surg.*, vol. 80, no. 6S Suppl 6, pp. S398–S402, Jun. 2018, doi: 10.1097/SAP.0000000000001345.

[33] Retsinformation, "Lov om et videnskabsetisk komitesystem og behandling af biomedicinske forskningsprojekter," *30/05/2003*, 2003. https://www.retsinformation.dk/eli/lta/2003/402 (accessed Apr. 19, 2021).

Figure legends

Figure 1: Illustration of the trial intervention. The trial drug contains 1000 mg vancomycin, 1000 mg cefazolin and 80 mg gentamicin dissolved in an infusion bag containing 500 ml of sterile isotonic saline. The placebo solution consists of 500 ml sterile isotonic saline.

Figure 2. Overview of the trial design. The patients are randomized to antibiotic treatment or placebo applied directly onto the breast implant and in the dissected tissue pocket. Patients who undergo bilateral breast reconstruction are randomized to antibiotics on one side and placebo on the contralateral side. Patients who undergo unilateral breast reconstruction are randomized to either antibiotics or placebo.

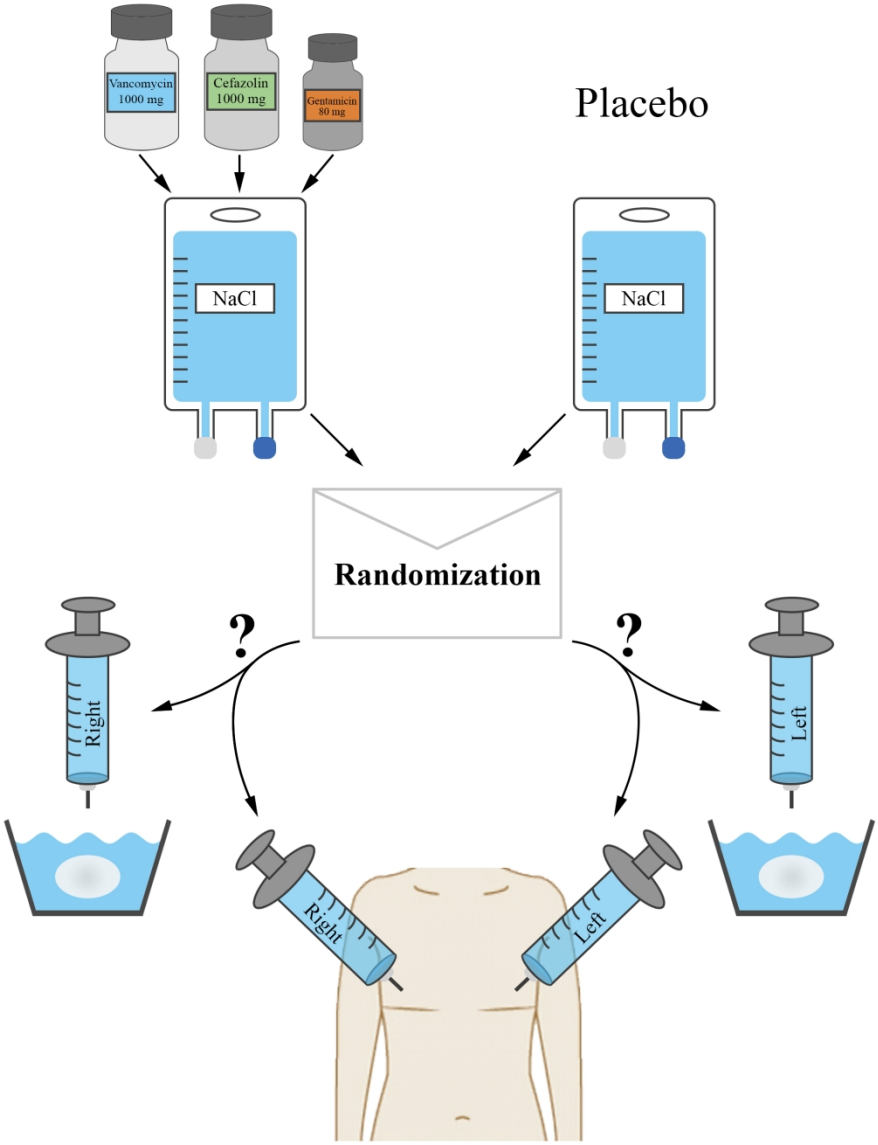


Figure 1: Illustration of the trial intervention. The trial drug contains 1000 mg vancomycin, 1000 mg cefazolin and 80 mg gentamicin dissolved in an infusion bag containing 500 ml of sterile isotonic saline. The placebo solution consists of 500 ml sterile isotonic saline.

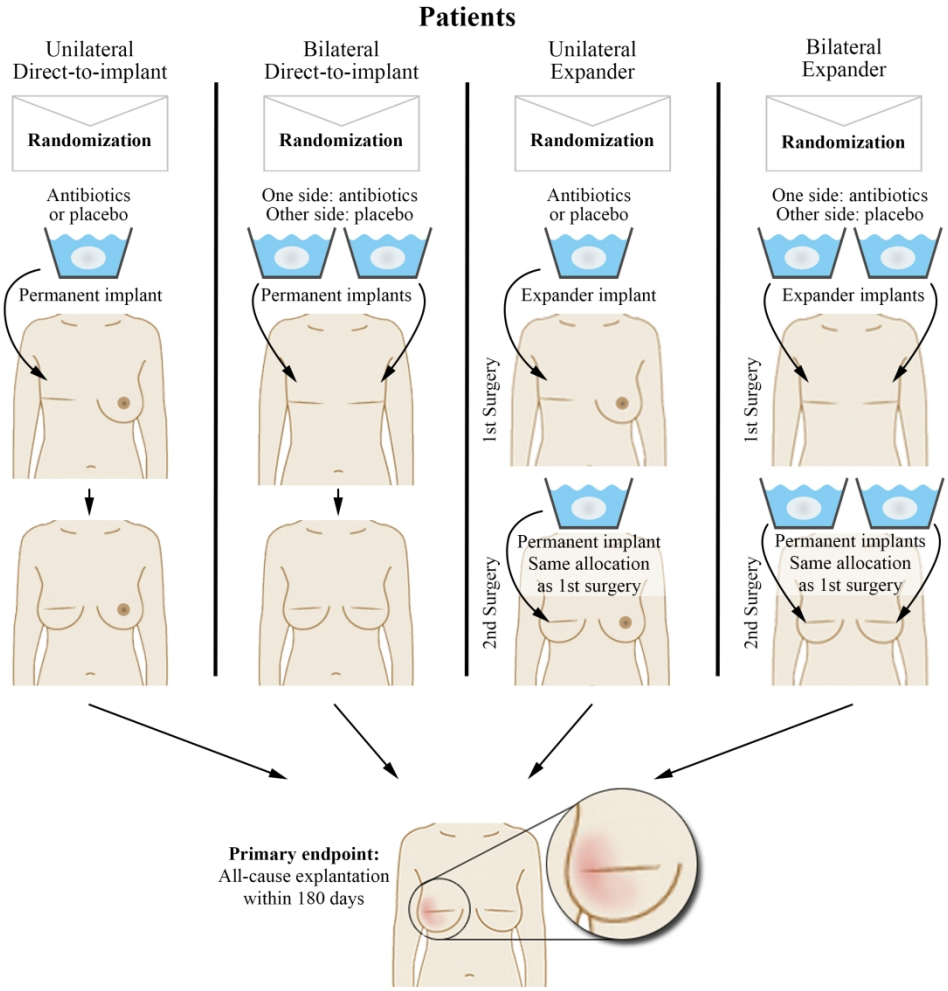


Figure 2. Overview of the trial design. The patients are randomized to antibiotic treatment or placebo applied directly onto the breast implant and in the dissected tissue pocket. Patients who undergo bilateral breast reconstruction are randomized to antibiotics on one side and placebo on the contralateral side. Patients who undergo unilateral breast reconstruction are randomized to either antibiotics or placebo.

Version 2.8
May 10, 2022

Trial Protocol

Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Short Title: Local Antibiotics for Breast Implants

Acronym: The Breast-AB Trial

By

Mikkel Herly, MD
Mathilde Hemmingsen, MD
Andreas Larsen, BMSc
Tim Weltz, BMSc

Version: 2.8 (10/5/22)

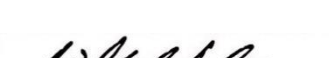
Sponsor Protocol Code Number: BREAST-AB-01

EudraCT number: 2020-002459-40

Date of Registration: 05-12-2020

Funding number: Novo Nordisk Foundation 0058322

Coordinating Sponsor-Investigator:
Mikkel Herly, MD
Department of Plastic Surgery and Burn Treatments
Blegdamsvej 9, 2100 Copenhagen
Phone: +45 35 45 33 53

Date: 10/05/22 Signature: 

Version 2.8
May 10, 2022

Trial synopsis

Title: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Lay title: Local Antibiotics for Women Undergoing Breast Reconstruction Surgery with Implants

Acronym: The BREAST-AB Trial

Trial design: A multi-center, investigator initiated, 1:1 randomized, double blind, placebo-controlled trial

Intervention: Application of gentamicin, vancomycin and cefazolin in a saline solution onto the implant and the dissected breast pocket used for breast reconstructive surgery

Objective: To determine the efficacy of local antibiotics in decreasing all-cause implant explantation

Inclusion criteria: Age ≥ 18, female, signed informed consent, breast reconstruction with implants including immediate/delayed reconstructions, bilateral/unilateral reconstructions and with or without flap reconstruction

Exclusion criteria: Pregnancy, breast feeding, known allergy towards any of the applied antibiotics, known anaphylactic reaction towards the same class of antibiotics as used in the trial, known allergy towards neomycin, known impaired renal function with GFR < 60 ml/min, participation in investigational drug trials and projects concerning disinfecting agents in the implant pocket and myasthenia gravis disease

Primary outcome: All-cause explantation of the breast implant within 180 days after the breast reconstruction surgery

Secondary outcomes:

- Time to explantation (days)
- All-cause explantation of the breast implant within 1 year after the breast reconstruction surgery (Y/N)
- Revision surgery with incision of the fibrous capsule within 180 days after the breast reconstruction surgery (Y/N)
- Revision surgery with incision of the fibrous capsule within 180 days after the breast reconstruction surgery (Y/N)
- Exchange of permanent implant to expander implant within 180 days after the breast reconstruction surgery (Y/N)
- Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction surgery (Y/N)

Tertiary outcomes: Assessed for patients undergoing unilateral breast reconstruction

- Time from surgery to discharge (days)

- Re-admission within 180 days after the surgery (Y/N)

Long-term outcomes: All-cause incision of the fibrous capsule and capsular contracture after 5, 10 and 15 years

Sample size: A total number of 1274 breasts undergoing breast reconstruction will be included in the trial. Assuming that 27 % of the patients undergo bilateral breast reconstruction, this entails 1003 included patients

Trial duration: 4 years and 180 days

Randomization: Stratified randomization according to the following factors:

- Unilateral or bilateral reconstruction
- Immediate or delayed reconstruction
- Previous or scheduled radiotherapy within the follow-up period (yes/no)

All patients undergoing unilateral breast reconstruction will be randomized to the trial drug or placebo in a ratio of 1:1. All patients undergoing bilateral reconstruction will be randomized to the trial treatment on one of their breasts and placebo to the contralateral breast. Combining these factors gives a total of 14 randomization strata per trial site. An allocation sequence will be made for each stratum and assign treatment in a fixed block size of two to ensure that the investigational drug and placebo is evenly distributed within each stratum

Treatment: The intervention treatment will consist of 1000 mg vancomycin (bactocin), 2 mL of 40 mg/mL gentamicin (hexamycin) and 1000 mg cefazolin (cefazolin "MIP") in a 500 mL sterile isotonic (9 %) saline solution. The placebo solution will consist of 500 mL of sterile isotonic (9%) saline. During the surgery, the responsible nurse will draw three 50 ml syringes from the infusion bag (in total 150 ml) and use it to wash the dissected implant pocket. Another 50 ml syringe will be drawn from the same infusion bag and used to wash the implant with the assigned solution prior to insertion in the implant pocket

Clinical follow-up: The included patients will adhere to the standard follow-up program according to the guidelines of the local treatment site

Blinding: The patients, surgeons and data assessors will be blinded to the treatment allocation throughout the trial period. The coordinating sponsor-investigator will be responsible for monitoring adverse events. The trial coordinating unit will have access to the randomization sequences. They will not take part in any treatment of the participants or analysis of data. In the case of emergency unblinding the trial coordinating unit can always be contacted

Safety: Treatment-related adverse events will be reported and assessed continuously throughout the trial period

Version 2.8
May 10, 2022

Trial Steering Committee

Thomas Bjarnsholt, Civil Engineer, DMSc, PhD
Professor at the Department of Microbiology, Copenhagen University Hospital, Rigshospitalet and Head of Costerton Biofilm Centre, SUND, University of Copenhagen

Tine Engberg Damsgaard, MD, PhD
Professor at the Department of Plastic Surgery and Burns Treatment,
Copenhagen University Hospital, Rigshospitalet

Peter Viktor Vester-Glowinski, MD, PhD
Medical Doctor and post doc at the Department of Plastic Surgery and Burns Treatment,
Copenhagen University Hospital, Rigshospitalet

Mikkel Herly, MD
Medical Doctor at the Department of Plastic Surgery and Burns Treatment,
Copenhagen University Hospital, Rigshospitalet

Søren J Sørensen, Cand. Scient., PhD
Professor and Head of the Section of Microbiology, Institute of Biology,
University of Copenhagen

Trial Coordinating Unit

Mathilde Nejrup Hemmingsen, MD
Department of Plastic Surgery and Burns Treatment
Copenhagen University Hospital, Rigshospitalet

Andreas Larsen, BMSc
Department of Plastic Surgery and Burns Treatment
Copenhagen University Hospital, Rigshospitalet

Tim Kongsmark Weltz, BMSc
Department of Plastic Surgery and Burns Treatment
Copenhagen University Hospital, Rigshospitalet

Version 2.8
May 10, 2022

Data Assessment Committee

Mathias Ørholt, MD
Department of Plastic Surgery and Burns Treatment
Copenhagen University Hospital, Rigshospitalet

Sebastian Wiberg, MD
Department of Anesthesiology and Intensive Care
Zealand University Hospital, Køge

An independent biostatistician who will be appointed before the assessment committee receives the data.

Version 2.8
May 10, 2022

Site Investigators	Clinical Trial Site
Lena Felicia Carstensen, MD	Department of Plastic Surgery, South-West Jutland Hospital, Finsensgade 35, 6700 Esbjerg +45 20 15 20 19
Mikkel Herly, MD	Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, +45 40 43 63 77
Lisbet Rosenkrantz Hölmich, Professor, MD, DMSc	Department of Plastic Surgery, Herlev and Gentofte Hospital, Borgmester Ib Juuls Vej 1, 2730 Herlev, +45 27 20 00 14
Vibeke Koudahl, MD, PhD	Department of Plastic Surgery, Hospital Little Belt, Kabbeltoft 25, 7100 Vejle +45 79 40 91 65
Volker-Jürgen Schmidt, Ass. Professor, MD	Department of Plastic Surgery, Zealand University Hospital, Sygehusvej 10, 4000 Roskilde
Camilla Bille, MD, PhD	Department of Plastic Surgery, Odense University Hospital, J. B. Winsløws Vej 4, 5000 Odense +45 65 41 24 45

Pharmaceutical manufacturer

Vancomycin
Bactocin®
MIP Pharma GmbH
Kirkeler Strasse 41
D-66 440 Blieskastel
Tyskland
Tlf: (+49) 68 42 96 09 0
Fax: (+49) 68 42 96 09 35
Email: info@mip-pharma.de
Website: www.mip-pharma.de

Cefazolin
Cefazolin "MIP"
MIP Pharma GmbH
Kirkeler Strasse 41
D-66 440 Blieskastel
Tyskland
Tlf: (+49) 68 42 96 09 0
Fax: (+49) 68 42 96 09 35
Email: info@mip-pharma.de
Website: www.mip-pharma.de

Gentamicin
Hexamycin
Sandoz A/S
Edvard Thomsens Vej 14
2300 København S
Tlf: 63951000
Fax: 63951001
Email: info.danmark@sandoz.com
Website: www.sandoz.dk

Natriumklorid
Natriumklorid "Fresenius Kabi" 9 mg/ml
Fresenius Kabi Denmark
Islands Brygge 57
2300 København S
Tlf: 33181600
Fax: 33181614
Emailadresse: info-dk@fresenius-kabi.com
Website: www.fresenius-kabi.com

Version 2.8
May 10, 2022

Table of contents

Version 2.8
May 10, 2022

1. Introduction

The incidence of breast cancer in Danish women is approximately 4700 per year.[1] Many women choose to undergo breast reconstruction with an implant following a mastectomy.[2] Unfortunately, implant-based breast reconstructions are associated with high complication rates. Postoperative infection of the breast and implant is one of the most severe short-term complications affecting around 5-10 % of the women.[3–7] Clinically infected implants must be surgically removed and the recovery period that follows is long and agonizing for the women. Subsequent attempts to reconstruct the breast are often postponed for several months or abandoned altogether.

Many strategies to prevent complications associated with bacterial contamination of the breast implant have been attempted.[8–13] According to a survey made by the American Society of Plastic Surgeons, the most widely followed approach is to apply antibiotics directly on the breast implant and the dissected tissue pocket to eliminate bacterial contamination during the surgery.[14] Although the use of local antibiotics on breast implants is now widespread, the treatment regimen has never been investigated in a randomized controlled trial.[15] This protocol will describe a randomized controlled trial that will investigate the effect of antibiotics applied locally on the implant and in the breast implant pocket on the incidence of infection that leads to explantation of the implant. The protocol has been designed in accordance with the SPIRIT 2013 Statement guidelines for protocol content.[16]

1.1. Bacterial contamination of the breast implant

The most prevalent bacterial agents associated with breast implant infections are similar to those of the breast duct and skin flora which suggests that these are possible sources of contamination.[17] The most common microorganisms found on infected breast implants are *Staphylococcus epidermidis* and *Cutibacterium acnes* (previously known as *Propionibacterium acnes*). Other bacteria that have been identified on breast implants are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.[18,19]

Previous studies propose that bacterial colonization of breast implants sometimes occur without any immediate clinical manifestations.[20,21] Instead, bacteria form a chronic, subclinical infection which is suspected to play a pivotal role in the development of a protracted immune reaction to the implant known as capsular contracture, affecting approximately 10-20 % of the patients.[22,23]

1.2. Local administered antibiotics

Local administration of antibiotics can achieve a high local concentration with a low systemic uptake[24] and thereby, minimize the systemic side effects while achieving high antibiotic penetrance. A high local concentration can ensure optimal effect of the antibiotics at the surgical site,[25] and thereby decrease the rate of postoperative surgical site infections, while potentially minimize the risk of antibiotic resistance. Local antibiotics also have the benefit of being independent from the tissue vascularization to achieve peak concentration as opposed to systemic antibiotics, which is an advantage during larger surgeries where the vascularization can be compromised.[25]

Studies have shown that the concentration of locally applied antibiotics in the surgical drain output is high during the first 24 hours[24] and after 72 hours, the concentration is negligible. Therefore, it is assumed that the potential side effects to the medication will occur within the first 72 hours and previous studies have not reported side effects to the local treatment.[26]

In 2001, Adams et al recommended an antibiotic regimen consisting of gentamicin, cefazolin combined with either bacitracin or vancomycin.[27] Internationally, this irrigation regimen has become the most commonly used for local breast pocket and implant irrigation.[28] In this trial we will investigate the combinations of gentamicin, cefazolin and vancomycin for irrigation for the breast pocket and breast implant.

1.3. Pre-clinical data

Preclinical data from in vitro models suggest that the combination of Gentamicin, Cefazolin and Vancomycin is the most efficient treatment against the bacterial species most commonly associated with breast implants.[27,29] Animal studies suggest that the local application of these antibiotics is safe.[30–33]

1.4. Clinical data

Current Evidence – a systematic review

The regimen of local antibiotics for breast implants and the dissected implant pocket has been widely applied in humans[14], but few studies have investigated the clinical effect. In May 2020, we searched scientific literature databases including Embase, Cochrane, Pubmed and Web of Science. We used the following search terms (((breast) AND (implant OR expander OR augmentation OR reconstruction)) AND (irrigation OR antibiotics OR antibacterial OR antiinfective OR antimicrobial OR disinfection OR bacitracin OR gentamicin OR vancomycin OR cefazolin OR neomycin)) AND (infection OR "capsular contracture" OR "capsular contraction" OR capsulitis). We included studies and reviews investigating the effect of any local antibiotics for irrigation of the implant and/or implant pocket in women undergoing implant-based breast reconstruction or cosmetic breast augmentation. Studies that did not list outcomes that were relevant for the primary and secondary outcomes of the BREAST-AB trial were excluded. The search identified 1697 studies of which 17 studies were included after title/abstract and full text screening. Seven review articles,[15,34–39] two prospective studies[40,41] and eight retrospective studies[41–49] were identified. No randomized controlled trials were identified. Most of the included studies included solely reported on patients undergoing cosmetic augmentation. Two studies included patients undergoing cosmetic breast augmentation and breast reconstruction, but they did not stratify the outcome.[48,49]

Two studies found a significant decrease in the infection rate when applying local antibiotics compared to a control group,[42,43] whereas one study found no significant decrease.[44] These studies were limited by the relatively small study populations and a poorly defined outcome and none of the studies were blinded. The rate of capsular contracture was found to be significantly decreased in two studies,[44,45] two studies found no significant decrease,[43,46] whereas one study found a significant increase in the capsular contracture rate.[41] However, all studies investigating

capsular contracture were limited by a short follow-up period for this long-term outcome. No adverse events have been reported in any of the included studies, and the local antibiotics were generally considered well-tolerated. See appendix 1 for a table of characteristics of the included studies.

1.5. Rationale

Administration of antibiotics directly on to the breast implant and dissected implant pocket will give a high concentration of the antibiotics where they are needed which may prevent bacterial contamination of the implant. This may decrease the rate of postoperative infections that lead to explantation of the implant and thereby improve the outcome for the patients.

1.6. Hypothesis

Local administration of gentamicin, cefazolin and vancomycin on the breast implant will decrease the rate of postoperative clinical infections compared to placebo.

2. Experimental design

2.1. Trial design

This trial is an investigator-initiated, randomized, double-blind and placebo-controlled clinical phase III trial. The triple antibiotic solution or placebo solution will be applied directly onto the implant used for breast reconstruction and the implant pocket. The included subjects who undergo bilateral reconstruction will be randomized to the triple antibiotic solution to one of their breasts and placebo to the contralateral breast. Those who undergo unilateral reconstruction will be randomized to the triple antibiotic solution or the placebo solution. See 4.2 for a more detailed description of the randomization. The triple antibiotic solution will consist of 1 g Vancomycin, 1 g Cefazolin and 80 mg Gentamicin diluted in 500 mL of saline.[27] The placebo solution will consist of 500 mL of saline. See section 5 for more information on the trial treatment.

2.2. Outcomes

2.2.1. Primary outcome

All-cause explantation of the breast implant within 180 days after the breast reconstruction surgery

Definition

All-cause explantation will be defined as explantation and discarding of the implant. Replacement of an expander with a permanent implant and replacement of a permanent breast implant with a new permanent breast implant due to cosmetic revisions such as asymmetry, implant malposition, change of size or implant rotation will not be counted as an explantation.

Rationale

The rationale for applying local antibiotics is to decrease the risk of severe complications associated with the presence of bacteria such as deep surgical site infection that leads to

explantation and discarding of the implant. Postoperative infection that leads to explantation of the implant will sometimes occur simultaneously with other complications where the cause of explantation may be unclear. Therefore, all-cause explantation is a logical and meaningful primary outcome.

The reason for excluding from the primary outcome: explantations of permanent implants followed by replacements with a new permanent implant for cosmetic reasons, is that such revisional surgery is not considered a proxy for severe complications that may be associated with a deep surgical site infection.

2.2.2. Secondary outcomes

The secondary outcomes will include:

- Time to explantation (days)
- All-cause explantation of the breast implant within 1 year after the breast reconstruction surgery (Y/N)
- Revision surgery with incision of the fibrous capsule within 180 days after the breast reconstruction surgery (Y/N)
- Exchange of permanent implant to expander implant within 180 days after the breast reconstruction surgery (Y/N)
- Surgical site infection that leads to antibiotic treatment within 180 days after the breast reconstruction surgery (Y/N)

Definition

Time to explantation will be defined as the amount of days between the breast reconstruction and the implant explantation surgery. The breast reconstruction surgery will be defined as the surgery where they received the allocated treatment. Surgical site infection will be defined according to the CDC classification of surgical site infetion[50] leading to antibiotic treatment with oral or intravenous antibiotics administered after the surgery.

Rationale

Time to explantation is important to determine the relation to the breast reconstruction surgery and the etiology of the event that leads to explantation of the implant. In some cases, the reconstructed breast may be upheld despite complications associated with bacteria by revisional surgery and therefore revisional surgery is an outcome of importance. Local antibiotics may decrease the incidence of postoperative surgical site infection requiring antibiotic treatment. Postoperative swelling and redness are to be expected after a larger surgery and can be difficult to distinguish from signs of infection. Therefore, surgical site infection that leads to antibiotic treatment is a logical outcome.

2.2.3. Tertiary outcomes

The tertiary outcomes will be assessed for patients undergoing unilateral breast reconstruction. The tertiary outcomes will include:

- Time from the breast reconstruction surgery to discharge (days)
- Re-admission within 180 days after the breast reconstruction surgery (Y/N)

Definition

Time to discharge will be defined as the amount of days between the breast reconstruction and the day of discharge. The breast reconstruction surgery will be defined as the surgery where the patient received the allocated treatment.

Rationale

The rationale for excluding bilateral patients from the tertiary outcomes is that all bilateral patients will receive placebo on one breast and the intervention on the contralateral breast. Therefore, patient related outcomes are only applicable for patients who undergo unilateral breast reconstruction. Postoperative infection that occurs during the hospital admission can prolong the admission period. Application of local antibiotics may shorten the admission period by decreasing the rate of postoperative infection occurring during the hospital admission. Infection that occurs after discharge can cause re-admission to the hospital. Local antibiotics may decrease the infection rate after discharge that require hospitalization.

2.2.3 Additional follow-up

The trial will include additional long-term outcomes focused on all-cause incision of the fibrous capsule around the breast implant, capsular contracture, Baker classification[51] and quality-of-life. See Gantt chart figure 1.

Definition

Capsular contracture and the Baker classification grade will be obtained from the National Patient Registry and the patients' medical journals after 5, 10 and 15 years. The BREAST-Q questionnaire[52] will be used to assess patient-reported outcomes. The patients will be contacted and asked to fill out the questionnaire with 5 year-intervals after the surgery.

Rationale

Previous studies suggest that bacterial contamination of the breast implant can occur without immediate clinical manifestation.[20,21] Instead, the bacteria form a chronic, subclinical infection which is suspected to play a pivotal role in the development of a protracted immune reaction to the implant called capsular contracture, affection 10-20 % of the patients.[22,23] The use of local antibiotics could potentially decrease the rate of capsular contracture by minimizing the bacterial contamination of the implant. Therefore, capsular contracture is a meaningful long-term outcome.

The application of local antibiotics may decrease the risk of postoperative complications and thereby decrease the risk of undergoing revision surgery. This in turn may lead to improved patient satisfaction and quality of life. Breast-Q is a validated tool used to quantify patient satisfaction and health-related quality of life after breast reconstruction surgery.[52]

Patients may be included in additional exploratory substudies at the time of implant explantation (e.g. expander removal). The exploratory substudies will be applied for in separate protocols to be approved by the relevant authorities and they will not interfere with this trial.

2.3. Setting and locations

The trial will be a nationwide multi-center trial with enrollment of patients from the following Danish hospitals:

- Department of Plastic Surgery and Burns Treatment, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen
- Department of Plastic Surgery, Herlev and Gentofte Hospital, Borgmester Ib Juuls Vej 1, 2730 Herlev
- Department of Plastic Surgery, Zealand University Hospital, Sygehusvej 10, 4000 Roskilde
- Department of Plastic Surgery, Odense University hospital, J. B. Winsløws Vej 4, 5000 Odense
- Department of Plastic Surgery, South-West Jutland Hospital, Finsensgade 35, 6700 Esbjerg
- Department of Plastic Surgery, Hospital Little Belt, Kabbeltoft 25, 7100 Vejle

All sites have clinical experience and expertise in performing implant-based breast reconstructions.

2.4. Number of Subjects

The trial will include patients until a total number of 1274 breast reconstructions according to our power calculation. We estimate that this number will be distributed on approximately 1003 patients provided that approximately 27% of patients undergo bilateral procedures. A total of 637 breasts will be allocated to placebo and 637 breasts will be allocated to treatment with the local antibiotic solution. The statistical considerations behind the sample size calculation is elaborated in section 8.1.

2.5. Trial Duration

We plan to begin inclusion in January 2021 at Rigshospitalet and Herlev Hospital. The other trial sites will begin enrollment thereafter according to the plan outlined below. We expect to begin inclusion at Zealand- and Odense University Hospital in spring 2022 followed by South-West Jutland Hospital and Hospital Little Belt in the autumn 2022. Additional trial sites may be applied for during the trial period if we do not meet our expected aim for included patients. See Gantt chart in figure 1.

We expect to include the 1003 patients over a 4-year period with planned completion January 2025, hence the last follow-up after 180 days will be completed after in July 2025. Currently, 700 women undergo reconstruction with implants each year in Denmark.[53] Therefore, we assume that it will be feasible to include approximately 334 patients per year.

Trial site	Inclusion period	Expected no. of included patients
Rigshospitalet	Jan 2021 – Jan 2025	274
Herlev Hospital	Jan 2021 – Jan 2025	253
University Hospital Zealand	Feb 2022 – Jan 2025	162
Odense University Hospital	April 2022 – Jan 2025	162
South-West Jutland Hospital	Oct 2022 – Jan 2025	76
Hospital Little Belt	Oct 2022 – Jan 2025	76
Total		**1003**

3. Subjects eligibility

All trial candidates will be evaluated for suitability by a medical doctor with expertise in the field of breast reconstruction surgery. All potential participating patients will receive oral information by the medical doctor and all information material will be given to the patient before the written informed consent form is signed. See participant timeline in figure 2.

3.1. Inclusion criteria

The patients must fulfill all the following criteria to be eligible for inclusion in the trial:

- Age ≥ 18 years
- Biologically female
- Signed informed consent
- Scheduled for breast reconstruction with implants or expanders including:
    a. Immediate or delayed reconstructions
    b. Bilateral or unilateral reconstructions
    c. With or without simultaneous flap reconstruction

3.2. Exclusion criteria

Patients are considered ineligible if any of the following criteria is fulfilled:

- Pregnancy
- Breast feeding
- Known allergy towards Vancomycin, Gentamicin and Cefazolin
- Known anaphylactic reaction towards other beta-lactam antibiotics or aminoglycosides
- Known allergy towards neomycin
- Known impaired renal function with GFR < 60 mL/min
- Participation in investigational drug trials and projects concerning disinfecting agents in the breast implant cavity
- Myasthenia Gravis

3.3. Pregnancy

Fertile women with child-bearing potential must provide a negative urine HCG prior to inclusion in the trial.

4. Enrollment

The patients will be registered in the trial after providing written consent (via the written consent form or a digital signature). The registered patients are considered enrolled in the trial when they have received the treatment. Each step of the enrollment procedure is described below.

4.1. Registration

All patients scheduled for a preoperative visit concerning a breast reconstruction procedure will be screened for eligibility and recorded in the individual trial site's screening log (appendix 2). No personal data will be recorded in the screening log. The following variables will be registered in the screening: screening number, screening date, initials of the person conducting the screening, age of the patient, if available date of pre-operative visit and surgery, eligibility of the patient yes/no and if "no", reason for non-eligibility. All patients who are considered eligible and have provided a written consent will be registered in the trial with a letter code for each site (e.g. RH for Rigshospitalet) combined with a record ID. The record ID will be assigned in sequential order as subjects are registered (1, 2, 3). Registration will include date of registration, central registration number, unilateral or bilateral reconstruction, type of surgery (immediate or delayed reconstruction) and radiotherapy status. The identification number remains constant throughout the trial.

4.2. Randomization and treatment assignment

Registered subjects will be randomized to placebo or the trial drugs on the day of surgery or the day before, and they will be considered enrolled in the trial when they have received the trial treatment. The randomization number will be the same as the record identification number. All patients undergoing unilateral breast reconstruction will be randomized to the trial drug or placebo in a ratio of 1:1. All patients undergoing bilateral reconstruction will be randomized to the trial treatment on one of their breasts and placebo to the contralateral breast (Investigational Product Dosage and Administration, section 5). See figure 3.

We will use a stratified randomization to ensure that potential risk factors which could confound the outcome are evenly distributed in the placebo and intervention group. The randomization strata will be generated by the following factors:

- Unilateral or bilateral reconstruction
- Immediate or delayed reconstruction
- Previous radiotherapy and/or planned radiotherapy within the follow-up period (yes/no)

When the three factors are combined, we get a total of 14 randomization strata per trial site. See appendix 3 for an overview of the 14 randomization strata. An allocation sequence will be made for each stratum and assign treatment in a fixed block size of two to ensure that the investigational drug and placebo is evenly distributed within each stratum. The fixed block size of two will not increase the risk of the investigator anticipating the allocation because the investigators are blinded to the treatment throughout the trial.

A member of the trial coordinating unit will access the computer-generated allocation sequence via RedCap The allocation will be registered in a REDCap module only available for members of the trial coordinating unit, who are unblinded.

4.2.1. Treatment assignment in two-stage breast reconstruction

Most implant-based breast reconstructions are performed in a single surgery with a permanent breast implant. During the surgery, the surgeon evaluates the quality and vitality of the dissected skin flaps before inserting the breast implant. In some patients, the skin quality is not considered suitable to allow for insertion of the permanent implant. These patients will be reconstructed in two stages, where an expander implant is used in the first surgery. The expander implant is used to expand the tissue before it can be replaced with a permanent implant after approximately 3 to 9 months of expansion. During the first surgery where the expander implant is inserted, the patient will be assigned to treatment according the randomization stratum. The allocation sequence number will be registered. At the second surgery where the expander is replaced with the permanent implant, the same treatment allocation will be used (e.g., if the right breast received treatment with the antibiotic solution in the first surgery, the right breast will receive treatment with the antibiotic solution in the second surgery). See figure 3.

4.2.2. Treatment assignment if a unilateral patient later becomes bilateral

In some cases, a unilateral patient can switch to the bilateral set-up. An example could be that a patient develops unilateral breast cancer, undergo mastectomy and reconstruction and then later in the trial period decide to undergo prophylactic risk-reducing mastectomy and reconstruction of the other breast. In this case, the patient would be assigned to either placebo or the trial drug in the first surgery. Then, when the patient undergo the second prophylactic surgery on the other breast, she will be allocated to receive placebo, if she had received antibiotics in the first surgery and vice versa, if she had received placebo in the first surgery she would be allocated to antibiotics on the other breast in the second surgery.

4.3. Registration failures

Registered subjects who are ineligible for randomization will be recorded as screening failures and they will be registered along with the reason for exclusion.

4.4. Discontinuation from the trial

Patients who withdraw their informed consent at any point during the trial period will be omitted from the trial and registered as “withdrawal of consent”. The coordinating sponsor investigator may omit participants from the trial at any point during the trial period due to safety of the participant. There will be no additional follow-up or data collection from these patients. The trial treatment is administered as a single dosage and therefore, exclusion will not have any effect on the trial treatment. The data analysis in the end of the trial will be performed on a modified intention-to-treat population, defined as all randomized patients with a valid informed consent.

4.4.1. Registration of dropouts

A designated representative at each trial site will be responsible for contacting the trial coordinating unit in case an included subject withdraws consent or is unable to complete the follow-up. All excluded patients will be recorded including date, central registration number, reason for exclusion and treatment allocation. Patients excluded from the trial will continue to follow the scheduled follow-up visits as a part of the standard treatment. Exclusion from the trial will not interfere with the standard treatment or entail any additional procedures or follow-up visits. Dropout rates will be monitored continuously by the sponsor-investigator.

4.4.2. Replacement of dropouts

Patients who drop out of the trial after enrollment (allocation to trial treatment) will not be replaced. The sample size calculation accounts for a drop-out rate of 5 %. In case of a drop-out rate of more than 5 %, we will apply imputations by chained equations and repeat the primary analysis (for the primary outcome) after imputations.

5. Treatment procedures

The trial drug will be administered as a single dosage during the breast reconstructive surgery. The administration procedure will be identical for the antibiotic solution and placebo. Only qualified healthcare personnel will perform the administration of trial drugs and no self-administration will take place. All personnel that handles investigational products will be instructed by members of the trial coordinating unit.

5.1. Investigational drugs

Gentamicin: 40 mg/ml gentamicin sulfate, 2 mL suspension in glass ampoules containing clear, colorless suspension without visible particles. Gentamicin is a broad-spectrum, bactericidal aminoglycoside primarily targeting gram-negative rods. Gentamicin (Hexamycin) produced by Sandoz A/S can be used but other producers of the same drug may be used as an alternative.

Cefazolin: 2096,72 mg cefazolin natrium equivalent to 2000 mg cefazolin of white or almost white powder in a capped vial. Cefazolin is a bactericidal antibiotic targeting both gram-negative and gram-positive bacteria. Cefazolin produced by MIP Pharma GmbH can be used but other producers of the same drug may be used as an alternative.

Vancomycin: one capped vial contains vancomycin hydrochloride equivalent to 1000 mg vancomycin as a finely grounded white powder with a pink to brown nuance. Vancomycin is bactericidal and targets gram-positive bacteria. Vancomycin (Bactocin®) produced by MIP Pharma GmbH can be used but other producers of the same drug may be used as an alternative.

All investigational drugs will be purchased through each trial site's clinical pharmaceutical services and their respective purchasing agreements. The investigational drugs will be kept and prepared in accordance with the manufacturer's recommendations (see 'summery of product characteristics' for cefazolin, gentamicin and vancomycin. The investigational drugs are part of the standard drug selection available at all trial sites. Therefore, we will not account for the overall stock of medicine.

However, we will account for the use of investigational medicine for each patient enrolled in the trial, including batch-number, expiration date, patient registration number and date of administration.

5.2. Preparation of the drug solutions

The preparation of the trial drug solution will take place in a medication room on the trial site on the day of the surgery or day before the surgery. A designated trained nurse will be responsible for the preparation and labelling of the trial drug solution. To minimize errors, it will be double checked. The labelling will include the trial identification number and marking of which breast the treatment will be applied to (right or left). The manufacturing nurse will contact the trial coordinating unit to confirm his/her identity as investigational drug manufacturer and obtain instruction as to how to allocate the trial treatment. The communication between the Trial coordinating unit and the manufacturing nurse will be looped to prevent mistakes regarding the allocation. A designated person will deliver the prepared solutions to the operation room, and the surgeon and the scrub nurse will be blinded for the allocation. A local SOP describing the preparation of the trial drugs will be compiled for each trial site (See appendix 4 for the SOP).

5.2.1. The antibiotic solution

The antibiotic solution will contain 1000 mg vancomycin, 80 mg gentamicin and 1000 mg cefazolin. Two syringes of 20 mL sterile saline will be drawn from an infusion bag containing 500 mL of sterile isotonic (9%) saline. The drawn saline will be infused in the capped vials containing cefazolin and vancomycin to dissolve the powder. The entire content of the vial containing vancomycin (20 mL) will be drawn back into the syringe and reinfused in the infusion bag. Only 10 mL of the cefazolin solution will be drawn from the capped vial containing the dissolved cefazolin. The 10 mL will also be reinfused in the infusion bag. The remaining content the capped vial will be discarded as medical waste. Hereafter, the 2 mL gentamicin suspension will be drawn into a syringe and infused in the infusion bag already containing vancomycin and cefazolin. See figure 4 for an illustration of the treatment preparation.

5.2.2. The placebo solution

The placebo solution will consist of 500 mL of sterile isotonic (9%) saline contained in a similar infusion bag.

Both the antibiotic solution and the placebo solution will be achromatic and will be indistinguishable from one another to ensure blinding of the health care personnel administering the drugs.

5.3. Investigational product administration

The assigned solution will be administered in an enclosed infusion bag. During the surgery, the responsible nurse will draw three 50 ml syringes from the infusion bag (entailing 150 ml) and use it to wash the dissected implant pocket. Another 50 ml will be drawn from the same infusion bag and used to wash the implant with the assigned solution prior to insertion in the implant pocket. The rest of the content in the infusion bag will be discarded as medical waste. Patients undergoing unilateral breast reconstruction will receive the assigned solution in one infusion bag marked either left or right. The patients undergoing bilateral breast reconstruction will be assigned to the

antibiotic solution to one breast and placebo solution to the contralateral breast, and the allocation sequence will determine which breast (right or left) gets which solution. The assigned solution bags will be marked right and left. See appendix 5 for SOP.

5.4. Dosage adjustments

The dose of investigational products will be fixed. The drug will be administered as a single dose, and one time only. No continuous administration will occur. It will not be possible to adjust the treatment after the administration of the treatment.

5.5. Concurrent medication

All included patients will be treated according to the local guidelines for breast reconstruction surgery at each trial site. The trial intervention will not interfere with any treatment procedures or administration of medication.

5.6. Blinding

The trial will double-blind so that the patients, site investigators and data monitors will be blinded to the allocation. Only the designated nurses and the members of the trial coordinating unit (who provide the randomization number and treatment allocation) are not blinded to the allocation. The unblinded persons will not in any way be part of the treatment, clinical evaluation of outcomes or data assessment.

The intervention drug will be prepared in infusion bags that will be indistinguishable to the placebo solution (infusion bags with saline). Both solutions are colorless and identical in appearance without any identifying features, and therefore we do not anticipate any risk of unintentional unblinding. The designated manufacturing nurse will contact the trial coordinating unit to receive the allocation by telephone. The randomization number and the treatment allocation will be provided by the trial coordinating unit. An emergency telephone number to the trial coordinating unit will be available to access the treatment allocation of individual patients in the case where emergency unblinding is necessary. If unblinding should occur, it will be documented via the case report form. The unblinded patient will not be excluded from the trial.

The patients will remain blinded until the end of the additional follow-up period. The rationale for keeping the patients blinded is to minimize bias when assessing the long-term outcomes. The patients can be unblinded upon request if they withdraw their consent to participate in the trial.

5.6.1. Ensuring blinding

The randomization number will ensure that the allocation is given during both surgeries in situations where the intervention treatment is repeated, for instance in two stage breast reconstruction (see section 4.2.1.) and if a unilateral patient becomes bilateral (see section 4.2.2.). During the first surgery, the registration number which equals the randomization number will be registered in the case report form. During the second surgery, the manufacturing nurse will contact the trial coordinating unit to obtain the treatment allocation and members of the trial

coordinating unit (who are unblinded) will look up the patient and inform the manufacturing nurse of the treatment allocation. The manufacturing nurse will not take part in any treatment procedures.

6. Data collection

The site investigators, along with members of the trial coordination unit, will be responsible for trial related data collection and entry. The individual trial site investigator may delegate assignments to designated doctors, scholarship students or nurses registered in the site-specific delegation log. Limited trial specific data will be entered into a numbered case report form (see appendix 6) at the time of inclusion and the surgery. This along with the screening log will be the only source data and all additional data will be obtained from the patients' medical records. Data will be entered directly into REDCap.

6.1. Variables

All enrolled patients (i.e. patients who have been assigned to treatment) will be entered into the database. An overview of included variables is provided below.

6.1.1. Pre-surgery variables

Trial related variables

- Study ID
- Site (location)
- Unilateral or bilateral breast reconstruction
- Immediate or delayed breast reconstruction
- Prophylactic or cancer (including carcinoma in situ)
- Radiation therapy status (Y/N)
- Name
- CPR number
- Date and name of data collector

Patient demographics

- Height (cm)
- Weight (kg)
- Smoking (never, former, active)
- Alcohol consumption (units per week)
- ASA classification (class I-VI)
- Race

Comorbidities

Prescription medications

Oral or intravenous antibiotic treatment within 2 months up to the surgery (Y/N, name and dose of antibiotic)

Prior breast surgery (Y/N, type of surgery, date)

Radiation therapy (dose and fraction)

Chemotherapy (type, dose, duration, frequency and no of cycles)

Antihormonal therapy (type, dose and duration)

Antibody therapy (type, dose and duration)

6.1.2. Surgical variables

Trial related variables

Date and time of surgery

Randomization number

Direct-to-implant or expander breast reconstruction

Deviations from the protocol

Date and time of treatment administration

Date and name of the data collector

Surgery characteristics

Mesh (Y/N)

Drain (Y/N)

Operative time (hours, minutes)

Implant type (brand, volume, texture, serial no.)

Implant placement (prepectoral or subpectoral)

Type of mastectomy procedure (nipple sparring KHAC10 or skin sparring KHAC15)

Type of reconstruction (KHAE00, KHAE05)

Thickness of mastectomy flap (mm)

Pre- and perioperative medications

VAC (Y/N)

6.1.3. Post-surgery variables

Characteristics

- Date of discharge
- Time to drain removal
- Post-operative medication
- Hematoma (Y/N)
- Mastectomy flap necrosis (Y/N)
- Nipple-areola-complex necrosis (Y/N)
- Seroma (Y/N)
- Explantation (Y/N)
- Date of explantation
- Indication of explantation
- Surgical site infection (Y/N)
- Bacterial agent (culturing/PCR)
- Revisional surgery with incision of the fibrous capsule (Y/N)
- Date of revisional surgery
- Indication of revisional surgery
- Baker grading
- Local adverse event
  - Severity of event
  - Time of event
  - Treatment/action taken
- Exclusion/loss to follow-up
  - Reason for exclusion

6.2. Clinical follow-up

The included patients will adhere to the standard follow-up program according to the guidelines of the local treatment site. There will no trial specific clinical follow-up visits. The patients will be instructed to contact the local treatment site if they should experience adverse events after they have been discharged. Additionally, the patients will be instructed to contact us if they receive relevant treatment related to the reconstructed breast at another hospital than their primary treatment site or via their general practitioner within 180 days after the surgery.

6.3. Data quality and security

Each variable is clearly defined in the case report form. Each data field will be provided with a definition of the variable, category for categorial variables and units for continuous variables.

All relevant trial documents including the signed consent form for each patient will be stored in the trial master file in a secure, locked place at each individual site. Only the site investigator and designated personnel will have access. The trial has been approved by the Danish Data Protection Agency. The files will be stored for 25 years, after which they will be destroyed.

7. Assessments of safety and harm

Women receiving breast cancer treatment and subsequently undergo breast reconstructive surgery are in high risk of experiencing adverse events in relation with the surgical treatment and cancer. These adverse events will not be registered as trial drug-adverse events.

The trial drugs are widely used internationally, and the adverse reaction profile for each drug is well-defined. (See 'Summery of Product Characteristics' section 4.8 for each drug). Previous studies show, that only low levels of locally administered antibiotics enter the bloodstream and therefore, the systemic side effects to the trial drugs are expected to be negligible.[54]

7.1. Expected adverse events unrelated to the trial drug

The complication rate following breast reconstruction is relatively high, and certain adverse events are to be expected after surgical resection of the breast and reconstruction with an implant. Several postoperative adverse events are likely to occur in the included patients due to the surgery and patient comorbidities and these events will not be registered as adverse events related to the interventional drugs. The following adverse events are expected in the included patients and will not be registered as adverse events to the trial treatment:

- Surgery specific complications: surgical site infection, implant malposition, expander deflation, expander port malfunction, implant/expander exposure, implant/expander rupture, wound dehiscence, hematoma, flap necrosis, seroma, capsular contracture, nerve damage, pain and lymphedema.
- Infectious disease including sepsis without signs of surgical site infection and fever without signs of surgical site infection.
- Cardiovascular disease including stroke, acute myocardial infarction, heart failure, arrythmia, cardiac arrest, pulmonary embolism, DVT and coagulopathy.
- Gastrointestinal disease including diarrhea, nausea, vomiting, gastroenteritis, colitis and ileus.
- Adverse events related to the kidneys and urinary tract including uremia, proteinuria, hematuria, interstitial nephritis, upper and lower urinary tract infection and pre-renal and post-renal kidney failure.
- Liver- and biliary tract disease including increase in serum liver enzymes levels, increase in bilirubin and alkaline phosphatase, hepatitis, liver cirrhosis, cholecystitis and pancreatitis.

- Respiratory disease including dyspnea, pleural effusion, pneumonia, pharyngitis, sinusitis and rhinosinusitis.
- Neurological disease including seizures, dizziness, impaired vision and vertigo.
- Metabolic disease including hyperglycemia and hypoglycemia.
- Immunological disease including thrombocytopenia, leukopenia, granulocytopenia, agranulocytosis, pancytopenia, leukocytosis, granulocytosis, anemia and polycythemia.
- Psychiatric disease including depression.
- Cancer recurrence.

7.2. Adverse events possibly related to the trial drug

The presumed relation to the trial drug will be evaluated using the 'Summery of Product Characteristics' for Gentamicin, Cefazolin and Vancomycin.

Examples of events that are likely to be related to the trial drugs are:

Erythema multiforme

Urticaria

Angioneurotic edema

Toxic epidermal necrolysis

Steven Johnsons' syndrome

Anaphylactic shock

Red man syndrome (appearing after a maximum of 10 minutes after administration)

Acute tinnitus

Acute deafness

Drug induced-acute kidney injury

Myasthenia gravis-like syndrome

Local adverse events (incl. surgical site infection, skin irritation, erythema, delayed wound healing, itching)

7.3. Adverse Event Reporting

Local antibiotics therapy is considered safe.[24] Previous studies have shown that the serum level of antibiotics after local application is low,[26] and therefore the risk of systemic organ toxicity is low. Gentamicin, cefazolin and vancomycin have all been approved for marketing for many years and have a well-known systemic adverse reaction profile. The combination of gentamicin, vancomycin and cefazolin has been used for local breast pocket irrigation for many years and is considered

safe.[36] Due to the extremely low systemic uptake when using locally administered antibiotics, the event of systemic adverse events following the trial intervention is considered very unlikely.

Adverse events related to the study drug will be assessed continuously by the co-investigator at each study site during the admission period (typically 72 hours). The trial drugs have a short half-life period of maximum 6 hours and will only be administered one time during the surgery. Therefore, it is considered very unlikely that adverse events related to the trial drugs should occur after discharge. If the patients experience adverse events, the site investigator will be responsible for registering the adverse event directly in the case report form. The patients will be instructed to contact the local treatment site if they should experience adverse events between the scheduled follow-up visits. The site investigator will report all adverse events to the coordinating sponsor-investigator who will be responsible for monitoring the safety of the trial. Each adverse event will require the investigator to fill in the AE form including the following variables: patient identification number, description of event, onset and end of event, severity, relation to the intervention, action taken and outcome.

Any adverse events occurring during the trial will be treated and monitored according to the local guidelines.

7.3.1. Adverse Events (AE) and Adverse Reactions (AR)

Any event that occurs after administrations of the trial drug regardless of the relation to the trial drug will be defined as an adverse event. Adverse reactions will be defined as events that are related to the trial drug.

7.3.2. Serious Adverse Events (SAE) and Serious Adverse Reactions (SAR)

For each recording of adverse event, the event will be evaluated as to whether it was a serious adverse event. A SAE will be defined as an AE that is life threatening, results in death, requires prolonged hospitalization or results in significant disability.

The site investigator at each trial site will be responsible for contacting the coordinating sponsor-investigator in case of serious adverse events within 24 hours of awareness. The site investigator will record the event in the case report form.

All serious adverse reactions will be reported yearly to the Danish Medicines Agency and the Regional Ethics Committee by the sponsor-investigator during the study period of three years and 6 months. After last patient last visit, serious adverse events will no longer be reported annually to the Danish Medicines Agency and The Regional Ethics Committee, since the effect of the study drug is considered negligible after 30 hours, and it is unlikely that any serious adverse events related to the study drug would occur after three days.

During the long-term follow-up, serious adverse events will not be reported in the annual safety report (ASR). Serious adverse events will be registered in the trial and included in a final clinical study report after the last long-term follow-up. See Gantt chart figure 1.

7.3.3. Suspected Unexpected Serious Adverse Reactions (SUSARs)

A SUSAR is an unexpected and serious presumed reaction to the trial drug. Section 4.8 in the 'Summery of Product Characteristics' for each drug (cefazolin, gentamicin and vancomycin) will be used as the reference safety information to determine whether or not the serious adverse event is unexpected. The sponsor-investigator will be responsible for that all relevant information about SUSARS, which are fatal or life threatening, is recorded and reported to the Regional Health Ethics Committee and the Danish Health and Medicines Authority as soon as possible, and no later than 7 days after the sponsor-investigator has been informed of such an event. No later than 8 days after the reporting, the sponsor-investigator is responsible for informing the Regional Health Ethics Committee and the Danish Medicines Agency of relevant treatment initiated by the co-investigator or a doctor at the trial site. Any other SUSAR must be reported to the Regional Health Ethics Committee and the Danish Medicines Agency no longer than 15 days after the sponsor-investigator has been informed. All trial investigators will be informed by the coordinating sponsor-investigator in the event of a SUSAR. See Gantt chart figure 1.

7.3.4. Severity of Adverse Events

The severity of each adverse event suspected to be related to the trial drug will be graded accordingly

- Mild: transient symptoms, with no interference in normal daily activity
- Moderate: persistent symptoms, resulting in moderate inhibition of daily activity
- Severe: persistent symptoms, resulting in severe inhibition of daily activity

7.3.5. Relationship of AE to Trial Intervention

For each AE suspected to be related to the trial drug, the probability will be rated accordingly

- Probable: there is good reason and adequate documentation to assume causal relationship
- Possible: a causal relationship is likely and cannot be dismissed
- Unlikely: the event is most likely related to an etiology other than the intervention
- Unknown: causality is not assessable

7.3.6. Adverse Reactions reporting during long-term follow-up

The annual reporting of SAR will not apply during the long-term follow-up period. Serious adverse reactions that we learn of during the long-term follow-up period of 5, 10 and 15 years will be recorded and included in a final clinical study report after the last long-term follow up.

SUSARS will be reported continuously to the Danish Medical Agency throughout the long-term follow-up period. See Gantt chart in figure 1.

8. Statistical considerations

8.1. Sample size

The trial will be powered towards the primary outcome. Previous data[3] do not suggest that there is an overrepresentation of bilateral infections compared to the unilateral infection rate which suggests that infections may be randomly distributed. Therefore, we do not assume that infections are correlated between breasts within the same patient. However, due to the paired design in the patients that undergo bilateral reconstruction, any correlation between the individual patient's breasts will increase the statistical power of this trial. The independent sampling unit of this trial will be 'breast', and the trial will be powered towards 'number of breasts', so that the final number of included patients will depend on the proportion of patients undergoing bilateral breast reconstruction. The incidence of the primary outcome is reported at 10%. With an α-level of 0.05, the trial will have a power of 0.90 to detect an absolute risk reduction of 5% if 1158 'breasts' are included. We will include patients, until a total number of 1274 breasts have completed the follow-up period of 180 days to account for a dropout rate of 10%. We estimate that 1003 patients will be included in the trial if approximately 27% of the patients undergo bilateral breast reconstruction.

8.2. Statistical analysis plan

The statistical analyses will be conducted on a modified intention-to-treat population, defined as all randomized patients with a valid informed consent. Categorical variables will be presented as frequencies whereas normally distributed continuous variables will be presented as mean ± SD, and as median (25^{th} percentile-75^{th} percentile) if non-normally distributed. Differences in endpoints (including the primary outcome) between the treatment allocations will be analyzed with mixed effects models taking into account the correlation between breasts in patients undergoing bilateral surgery. The stratified randomization approach will ensure an even distribution of known outcome risk factors between the placebo group and the treatment group. Logistic regression models will be applied to compare the odds of the primary outcome between the two treatment groups. The models will be adjusted for potential confounders, including age, smoking, body mass index, indication for surgery (prophylactic mastectomy versus mastectomy after cancer diagnosis), immediate reconstruction versus delayed reconstruction, bilateral versus unilateral surgery, and radiotherapy status. In case of missingness greater than 5% for the primary outcome, we will apply multiple imputations by chained equations and repeat the primary analysis as sensitivity analysis. A statistical significance level of 0.05 will be applied throughout. The open source statistical program "R" (http://www.r-project.org) will be used for data treatment and statistical analysis.

8.3. Registration of changes

Changes to the original statistical analysis plan will be recorded in the sponsor's trial master file before unblinding.

9. Ethical considerations

This trial will be conducted in accordance with EU and national legislation on medical research in capable patient volunteers. Eligible subjects shall provide oral and written informed consent to participate in the trial. The informed consent can be withdrawn by the participant at any time during the trial after which the patient will convert to receiving treatment as determined by local guidelines. All data on trial participants will be protected according to the General Data Protection Regulation (GDPR), the data protection law and the Danish Health Act. The project is approved by the Danish Data Protection Agency and is to be approved by the Danish Medicines Agency and the Regional Committee on Health Research Ethics. The trial will be conducted according to national and international standards of good clinical practice (GCP) and will be monitored by the regional GCP unit.

9.1. Ethical justification

The trial will investigate the beneficial effects and potential side-effects of applying gentamicin, vancomycin and cefazolin locally onto the breast implant during breast reconstruction surgery. This may limit bacterial contamination of the implant and thereby decrease the risk of postoperative infection which is associated with a poor outcome for the patients. Therefore, participation in this trial could benefit the individual participant.

Inclusion in the trial may benefit the individual subject by decrease the risk of undergoing explantation of the implant, minimizing the risk of postoperative infection, minimize the hospitalization period and may as well improve the outcome for future women undergoing implant-based breast reconstructive surgery. Alternatively, we may find that the local antibiotics do not have a clinically relevant effect and perhaps negative side-effects that should be explored further.

The trial drug regimen is widely used internationally[14] but the potential positive and/or negative effects of using local antibiotics on breast implants have never been tested against placebo in a randomized trial. See appendix 1 for a review of the current literature.

Though the drug regimen has not been tested in a randomized controlled trial, the drugs have been applied by plastic surgeons for several years, and no adverse events has been reported.[42,44,48,55] Therefore, the drug regimen is expected to be of minimal risk to the subjects in the trial. Moreover, the systemic levels of antibiotics after locally applied antibiotics has been shown to be much lower than the levels seen with systemic antibiotics and the trial treatment consists of a single dose, and therefore we do not expect systemic adverse reactions to the trial drugs.[36]

Currently, no clinical guidelines exist in Denmark regarding the prophylactic use of local antibiotics to breast implants, and the treatment depends on the local approach at each hospital and the individual surgeon's preference. Allocation to placebo in this trial is therefore considered ethically acceptable. Application of local vancomycin on the breast implant has been used routinely at the Department of Plastic Surgery, Herlev and Gentofte Hospital, in the past years. This treatment regimen is not based on any evidence and is scientifically unjustified. Therefore, we find it ethically acceptable to include patients from Herlev and Gentofte Hospital in the trial. All eligible patients

from Herlev and Gentofte Hospital will receive this information before providing consent and specific written patient information material will be provided for these patients. Patients at Herlev and Gentofte Hospital who are not included in the trial will receive local vancomycin during the breast reconstruction surgery.

The results from this trial could change the guidelines for breast reconstruction surgery on an international level and be used to provide patients with evidence-based treatment.

9.2. Informed consent

Registered patients will receive information about the trial in their e-boks. They will be informed that the trial coordinating unit will contact them to provide oral information about the project after they have had time to read the information material about the trial. The contact information for registered patients will be passed on to the trial coordination unit from the treatment site.

Recruitment of trial participants will be carried out at the time of the preoperative patient visit or by telemedicine (i.e. telephone or a secure video connection) by a medical doctor with relevant expertise. All patients are encouraged to bring a third party (i.e. relative or partner) to this appointment. The responsible medical doctor can assign a designated nurse or medical student with relevant expertise to provide the patient with both oral and written information concerning the trial during this visit as the medical doctor has limited time available at the initial pre-operative consultation. The investigator carrying out the recruitment will be responsible for obtaining the informed consent (either by a digital consent or a written consent form) from the patients prior to any protocol-related activities. The conversation will take place in a private room behind a closed door. Participation in the trial will not influence the choice of treatment. The patient and the responsible medical doctor must personally provide a dated signature digitally or on a consent form. The informed consent can be withdrawn by the patient at any time and without explanation, after which the patient will receive the standard treatment according to the local guidelines. No personal data will be collected patients before informed consent is obtained.

9.2.1. General considerations

We will strive to provide the patients with at least 24 hours of consideration, but in some patients this will not be possible. For instance, when it comes to patients undergoing primary breast reconstruction, the breast reconstruction is performed during the same surgery as the cancer surgery and the patients are treated in an accelerated cancer treatment course. Due the short period of time from planning to carrying out the surgery, the decisions regarding possible use of implants or expander for reconstruction are often made a few hours before the surgery. In these cases, the patients will have at least 2 hours of consideration.

10. Direct access to source data/documentation

The site investigator will permit direct access to source data blinded for treatment allocation for monitoring, audits and reviews by the Health Ethics Committee, Good Clinical Practice unit, Danish Medicines Agency and other regulatory authorities.

11. Data management

All data management will be conducted according to guidelines of the Danish Data Protection Agency. The data will be kept in REDCap. The database will be maintained for 25 years from the last patient visit and anonymized when the approval from the Danish Data Protection Agency terminates.

12. Quality control and quality assurance

The trial will be conducted according to the approved protocol and will comply to standard procedures for quality control and quality assurance. The investigator at each trial site will report any deviations from standard protocol to the coordinating sponsor-investigator either by direct contact or via the case report form.

The trial conduct, data generation, data documentation and reporting will be in accordance with ICH-GCP guidelines and the trial will be monitored by the national Good Clinical Practice (GCP) unit. Monitoring will be conducted upon initiation of the trial, during the trial and at termination of the trial.

The sponsor-investigator, local trial site investigators and the trial coordinating unit are responsible for maintaining up-to-date accrual information, enrollment status and safety data. It is the responsibility of the sponsor-investigator to ensure oversight of trial related activities and on a yearly basis, report trial progression, enrollment status and safety data to the Trial Steering Committee (see Trial Steering Committee Charter). The sponsor-investigator will submit all relevant documents to the board members for scientific progress review. The sponsor-investigator is responsible for ensuring that access to clinical trial data is consistent with data protection principles and in accordance with the patients' informed consent provided in relation to their participation in the clinical trial.

The trial steering committee will be responsible for data monitoring and quality control of the data extracted from the patients' medical journals at the long-term follow-up after 5, 10 and 15 years.

13. Finance and insurance

The trial is funded by a project grant in surgical research of 2,975,000 DKK from the Novo Nordisk Foundation. The grant budget will cover a PhD salary, a part-time post-doc salary, and medicine and materials required for the trial. See attached research budget. The grant is disbursed to Professor Tine Engberg Damsgaard on behalf of Rigshospitalet and administered by the Financial Department of the Centre of Head and Orthopedics, Rigshospitalet. None of the researchers have any financial disclosures or relation to the Novo Nordisk Foundation. The Novo Nordisk Foundation have had no part in the design of the study, and they will not participate in the reporting of the results. The study participants will not be reimbursed for participation in the study. The trial participants are insured according to the Danish patient compensation scheme.

14. Publication plan

The trial is registered on EudraCT. The trial protocol will be registered at ClinicalTrials.gov before enrollment of the first patient. The protocol will be published prior to unblinding of the results in a methodology article including the statistical analysis plan. The main article will include the primary and secondary outcomes. The tertiary and long-term outcomes will be included in subsequent articles. The manuscripts will be used to report the results of the trial to the scientific community and will adhere to the CONSORT guidelines.[56] The members of the trial coordinating unit will be co-first authors and the coordinating sponsor-investigator will be senior/last author and corresponding author. All site investigators and members of the Trial Steering Committee will be co-authors. Other co-authorships will be decided by contribution according to the ICMJE authorship guidelines[57] depending on personal involvement. All publications will refer to the trial group which will include all contributing parties to the BREAST-AB trial. The manuscripts will be submitted to peer-reviewed international journals and both positive, negative and inconclusive results will be published. The findings of the trial will be shared with participating sites and presented at national and international conferences. The results will be disseminated to the public but will not be shared directly with participating patients. See Gant chart in figure 1.

15. Tasks and Responsibilities

Trial Coordinating Unit: Protocol development, daily management in the trial period, contact to Good Clinical Practice monitoring unit, contact to trial sites, data dictionary development, responsible for providing randomization sequences for each trial site, provide deidentified data to the trial steering committee for safety monitoring, available for unblinding the treatment allocation of individual patients in the case of an emergency, instructing health care personnel involved in the trial, data collection and management.

Coordinating Sponsor-Investigator: Overall responsibility for protocol development, funding, budget overview, ethical approval, trial registration, trial oversight (including enrollment trends and safety), contact to Good Clinical Practice monitoring unit, potential recruitment of additional sites, and dissemination and presentation of results.

Trial Steering Committee: Clinical and scientific advising to the sponsor-investigator, evaluation and recommendations based on yearly reports regarding enrollment trends, progress and safety of the trial, counseling regarding scientific reporting of data, data quality control during long-term follow-up. See 'Trial Steering Committee Charter' for a more thorough description of the tasks and responsibilities of the committee.

Site investigators: Responsible for site-specific enrollment, evaluation and reporting of eligible patients not included, education of personnel at trial sites, reporting of site-specific issues or challenges to the coordinating sponsor-investigator, inclusion of patients, responsible for obtaining informed consent, contact to the regional Good Clinical Practice monitoring unit, trial related data entry in the case report form

Clinical personnel: Preparation and administration of the trial drugs.

Good Clinical Practice-unit: See section 12.

Data Assessment Committee: Assessment and analysis of data.

16. Contact information

Contact for public queries

Mathilde Nejrup Hemmingsen, MD

Department of Plastic Surgery and Burns Treatment

Email: mathilde.nejrup.hemmingsen@regionh.dk

Phone: + 45 35 45 83 69

Contact for scientific queries

Mikkel Herly, MD

Department of Plastic Surgery and Burn Treatments

Blegdamsvej 9, DK2100 Copenhagen

E-mail: mikkel.herly@regionh.dk

Phone: +45 35 45 33 53

17. References

1. Bekæmpelse K. Statestik om brystkræft.
2. 2018 Plastic Surgery Statistics.
3. Coroneos CJ, Selber JC, Offodile AC, Butler CE, Clemens MW. US FDA breast implant postapproval studies: long-term outcomes in 99,993 patients. *Ann Surg*. 2019;269(1):30-36.
4. Stevens WG, Calobrace MB, Harrington J, Alizadeh K, Zeidler KR, d'Incelli RC. Nine-year core study data for Sientra's FDA-approved round and shaped implants with high-strength cohesive silicone gel. *Aesthet Surg J*. 2016;36(4):404-416.
5. Patrick Maxwell G, Van Natta BW, Bengtson BP, Murphy DK. Ten-year results from the natrelle 410 anatomical form-stable silicone breast implant core study. *Aesthetic Surg J*. 2015;35(2):145-155. doi:10.1093/asj/sju084
6. Petersen A, Eftekhari ALB, Damsgaard TE. Immediate breast reconstruction: A retrospective study with emphasis on complications and risk factors. *J Plast Surg Hand Surg*. 2012;46(5):344-348. doi:10.3109/2000656X.2012.700025
7. Dikmans REG, Negenborn VL, Bouman M-B, et al. Two-stage implant-based breast reconstruction compared with immediate one-stage implant-based breast reconstruction augmented with an acellular dermal matrix: an open-label, phase 4, multicentre, randomised, controlled trial. *Lancet Oncol*. 2017;18(2):251-258.
8. Chopra K, Gowda AU, McNichols CHL, Brown EN, Slezak S, Rasko Y. Antimicrobial Prophylaxis Practice Patterns in Breast Augmentation. *Ann Plast Surg*. 2017;78(6):629-632. doi:10.1097/sap.0000000000000942
9. Yalanis GC, Liu E-W, Cheng H-T. Efficacy and safety of povidone-iodine irrigation in reducing the risk of capsular contracture in aesthetic breast augmentation: a systematic review and meta-analysis. *Plast Reconstr Surg*. 2015;136(4):687-698.
10. Kenna DM, Irojah BB, Mudge KL, Eveler K. Absorbable antibiotic beads prophylaxis in immediate breast reconstruction. *Plast Reconstr Surg*. 2018;141(4):486e-492e. doi:10.1097/PRS.0000000000004203
11. Nava MB, Catanuto G, Rocco N. Role of Mitomycin C in Preventing Capsular Contracture in Implant-Based Reconstructive Breast Surgery: A Randomized Controlled Trial. *Plast Reconstr Surg*. 2017;140(6):823e-824e. doi:10.1097/PRS.0000000000003851
12. Hunsicker LM, Chavez-Abraham V, Berry C, McEwen D. Efficacy of vancomycin-based continuous triple antibiotic irrigation in immediate, implant-based breast reconstruction. *Plast Reconstr Surg - Glob Open*. 2017;5(12). doi:10.1097/GOX.0000000000001624
13. Tutela JP, Duncan DP, Kelishadi SS, Chowdhry S, Boyd T, Little JA. Continuous Postoperative Antibiotic Irrigation via Catheter System Following Immediate Breast Reconstruction. *Eplasty*. 2015;15:e49.
14. Hidalgo DA, Sinno S. Current trends and controversies in breast augmentation. *Plast*

Reconstr Surg. 2016;137(4):1142-1150.

15. Lynch JM, Sebai ME, Rodriguez-Unda NA, Seal S, Rosson GD, Manahan MA. Breast pocket irrigation with antibiotic solution at implant insertion: a systematic review and meta-analysis. *Aesthetic Plast Surg*. 2018;42(5):1179-1186.

16. Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med*. 2013;158(3):200-207.

17. Pittet B, Montandon D, Pittet D. Infection in breast implants. *Lancet Infect Dis*. 2005;5(2):94-106. doi:10.1016/S1473-3099(05)01281-8

18. Bartsich S, Ascherman JA, Whittier S, Yao CA, Rohde C. The breast: a clean-contaminated surgical site. *Aesthetic Surg J*. 2011;31(7):802-806. doi:10.1177/1090820X11417428

19. Ahn CY, Ko CY, Wagar EA, Wong RS, Shaw WW. Microbial evaluation: 139 implants removed from symptomatic patients. *Plast Reconstr Surg*. 1996;98(7):1225-1229. doi:10.1097/00006534-199612000-00016

20. Pajkos A, Deva AK, Vickery K, Cope C, Chang L, Cossart YE. Detection of subclinical infection in significant breast implant capsules. *Plast Reconstr Surg*. 2003;111(5):1605-1611.

21. Rieger UM, Mesina J, Kalbermatten DF, et al. Bacterial biofilms and capsular contracture in patients with breast implants. *Br J Surg*. 2013;100(6):768-774.

22. Benediktsson K, Perbeck L. Capsular contracture around saline-filled and textured subcutaneously-placed implants in irradiated and non-irradiated breast cancer patients: Five years of monitoring of a prospective trial. *J Plast Reconstr Aesthetic Surg*. 2006;59(1):27-34. doi:10.1016/j.bjps.2005.08.005

23. Calobrace MB, Stevens WG, Capizzi PJ, Cohen R, Godinez T, Beckstrand M. Risk factor analysis for capsular contracture: A 10-year sientra study using round, smooth, and textured implants for breast augmentation. *Plast Reconstr Surg*. 2018;141(4S):20S-28S. doi:10.1097/PRS.0000000000004351

24. White IV RR, Pitzer KD, Fader RC, Rajab MH, Song J. Pharmacokinetics of topical and intravenous cefazolin in patients with clean surgical wounds. *Plast Reconstr Surg*. 2008;122(6):1773-1779.

25. Berberich C, Sanz-Ruiz P. Risk assessment of antibiotic resistance development by antibiotic-loaded bone cements: Is it a clinical concern? *EFORT Open Rev*. 2019;4(10):576-584. doi:10.1302/2058-5241.4.180104

26. Sweet FA, Roh M, Sliva C. Intrawound application of vancomycin for prophylaxis in instrumented thoracolumbar fusions: efficacy, drug levels, and patient outcomes. *Spine (Phila Pa 1976)*. 2011;36(24):2084-2088.

27. Adams Jr WP, Conner WCH, Barton Jr FE, Rohrich RJ. Optimizing breast-pocket irrigation: the post-betadine era. *Plast Reconstr Surg*. 2001;107(6):1596-1601.

28. Epps MT, Langsdon S, Pels TK, et al. Pocket Irrigation and Technique During Reconstructive Surgery: An American Society of Plastic Surgery Survey of Current Practice. *Ann Plast Surg*.

2019;82(6S Suppl 5):S427-S432. doi:10.1097/SAP.0000000000001790

29. Adams WP, Conner WCH, Barton FE, Rohrich RJ. Optimizing breast pocket irrigation: an in vitro study and clinical implications. *Plast Reconstr Surg*. 2000;105(1):334-338.

30. Darouiche RO, Meade R, Mansouri MD, Netscher DT. In vivo efficacy of antimicrobe-impregnated saline-filled silicone implants. *Plast Reconstr Surg*. 2002;109(4):1352-1357. doi:10.1097/00006534-200204010-00022

31. Zebala LP, Chuntarapas T, Kelly MP, Talcott M, Greco S, Riew KD. Intrawound vancomycin powder eradicates surgical wound contamination: an in vivo rabbit study. *J Bone Joint Surg Am*. 2014;96(1):46-51. doi:10.2106/JBJS.L.01257

32. Yarboro SR, Baum EJ, Dahners LE. Locally administered antibiotics for prophylaxis against surgical wound infection. An in vivo study. *J Bone Joint Surg Am*. 2007;89(5):929-933. doi:10.2106/JBJS.F.00919

33. Cavanaugh DL, Berry J, Yarboro SR, Dahners LE. Better prophylaxis against surgical site infection with local as well as systemic antibiotics. An in vivo study. *J Bone Joint Surg Am*. 2009;91(8):1907-1912. doi:10.2106/JBJS.G.01237

34. Frois AO, Harbour PO, Azimi F, et al. The role of antibiotics in breast pocket irrigation and implant immersion: a systematic review. *Plast Reconstr Surg Glob Open*. 2018;6(9).

35. Drinane JJ, Chowdhry T, Pham T-H, et al. Examining the Role of Antimicrobial Irrigation and Capsular Contracture: A Systematic Review and Meta-analysis. *Ann Plast Surg*. 2017;79(1):107-114. doi:http://dx.doi.org/10.1097/SAP.0000000000001134

36. Campbell CA. The Role of Triple-Antibiotic Saline Irrigation in Breast Implant Surgery. *Ann Plast Surg*. 2018;80(6S Suppl 6):S398-S402. doi:10.1097/SAP.0000000000001345

37. Horsnell JD, Searle AE, Harris PA, et al. Intra-operative techniques to reduce the risk of capsular contracture in patients undergoing aesthetic breast augmentation - A review. *Surgeon*. 2017;15(5):282-289. doi:http://dx.doi.org/10.1016/j.surge.2017.03.001

38. Huang N, Liu M, Yu P, Wu J. Antibiotic prophylaxis in prosthesis-based mammoplasty: a systematic review. *Int J Surg*. 2015;15:31-37.

39. Samargandi OA, Joukhadar N, Al Youha S, Thoma A, Williams J. Antibiotic Irrigation of Pocket for Implant-Based Breast Augmentation to Prevent Capsular Contracture: A Systematic Review. *Plast Surg (Oakville, Ont)*. 2018;26(2):110-119. doi:10.1177/2292550317747854

40. Burkhardt BR, Dempsey PD, Schnur PL, Tofield JJ. Capsular contracture: a prospective study of the effect of local antibacterial agents. *Plast Reconstr Surg*. 1986;77(6):919-932.

41. Stevens WG, Nahabedian MY, Calobrace MB, et al. Risk factor analysis for capsular contracture: a 5-year Sientra study analysis using round, smooth, and textured implants for breast augmentation. *Plast Reconstr Surg*. 2013;132(5):1115-1123.

42. Araco A, Gravante G, Araco F, Delogu D, Cervelli V, Walgenbach K. Infections of breast implants in aesthetic breast augmentations: a single-center review of 3,002 patients.

Aesthetic Plast Surg. 2007;31(4):325-329.

43. Pfeiffer P, Jørgensen S, Kristiansen TB, Jørgensen A, Hölmich LR. Protective effect of topical antibiotics in breast augmentation. *Plast Reconstr Surg*. 2009;124(2):629-634.

44. Giordano S, Peltoniemi H, Lilius P, Salmi A. Povidone-iodine combined with antibiotic topical irrigation to reduce capsular contracture in cosmetic breast augmentation: a comparative study. *Aesthetic Surg J*. 2013;33(5):675-680.

45. Blount AL, Martin MD, Lineberry KD, Kettaneh N, Alfonso DR. Capsular contracture rate in a low-risk population after primary augmentation mammaplasty. *Aesthetic Surg J*. 2013;33(4):516-521.

46. Drinane JJ, Bergman RS, Folkers BL, Kortes MJ. Revisiting triple antibiotic irrigation of breast implant pockets: A placebo-controlled single practice cohort study. *Plast Reconstr Surg - Glob Open*. 2013;1(7). doi:10.1097/GOX.0b013e3182aa8760

47. Drinane JJ, Kortes MJ, Bergman RS, Folkers BL. Evaluation of antibiotic irrigation versus saline irrigation in reducing the long-term incidence and severity of capsular contraction after primary augmentation mammoplasty. *Ann Plast Surg*. 2016;77(1):32-36.

48. Adams WPJ, Rios JL, Smith SJ. Enhancing patient outcomes in aesthetic and reconstructive breast surgery using triple antibiotic breast irrigation: six-year prospective clinical study. *Plast Reconstr Surg*. 2006;117(1):30-36.

49. Gutowski KA, Mesna GT, Cunningham BL. Saline-filled breast implants: A Plastic Surgery Educational Foundation multicenter outcomes study. *Plast Reconstr Surg*. 1997;100(4):1019-1027. doi:10.1097/00006534-199709001-00028

50. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC Definitions of Nosocomial Surgical Site Infections, 1992: A Modification of CDC Definitions of Surgical Wound Infections. *Infect Control Hosp Epidemiol*. 1992;13(10):606-608. doi:10.1086/646436

51. Spear SL, Baker JJL. Classification of capsular contracture after prosthetic breast reconstruction. *Plast Reconstr Surg*. 1995;96(5):1119-1123; discussion 1124.

52. Pusic AL, Klassen AF, Scott AM, Klok JA, Cordeiro PG, Cano SJ. Development of a new patient-reported outcome measure for breast surgery: the BREAST-Q. *Plast Reconstr Surg*. 2009;124(2):345-353.

53. Landspatientsregisteret: Avanceret udtræk.

54. Zalavras CG, Patzakis MJ, Holtom P. Local antibiotic therapy in the treatment of open fractures and osteomyelitis. *Clin Orthop Relat Res*. 2004;427:86-93.

55. Samargandi OA, Joukhdar N, Shaikh OM, Al Youha S, Williams J. Role of Antibiotic Irrigation in Preventing Capsular Contracture and Other Complication After Breast Augmentation: A Systematic Review and Meta-analysis. *Plast Reconstr Surg Glob Open*. 2016;4(9 Suppl).

56. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. *Int J Surg*. 2011;9(8):672-677. doi:10.1016/j.ijsu.2011.09.004

57. International Committee of Medical Journal Editors (ICMJE). International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals: Sample References. 2009;88(May):1-19.

Version 2.8
May 10, 2022

18. Appendices

Appendix 1: Systematic review

Appendix 2: Screening log

Appendix 3: Randomization strata

Appendix 4: Standard operating form for preparation of investigational drugs

Appendix 5: Standard operating form for administration of investigational drugs

Appendix 6: Case report forms

Appendix 7: Medicine account

Appendix 8: Trial Steering Committee Charter

Figure 1: Gantt chart

Figure 2: Participant timeline

Figure 3: Patients

Figure 4: Preparation of treatment

EudraCT: 2020-002459-40
The BREAST-AB Trial
Samtykkeerklæring version 2.0

FORSKERS VERSION: underskrives og indhentes med skriftligt samtykke

Informeret samtykke til deltagelse i et sundhedsvidenskabeligt forskningsprojekt.

Titel: Forebyggende behandling med lokal antibiotika af brystimplantater til brystrekonstruktion

Originaltitel: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Erklæring fra forsøgspersonen:

Jeg har fået skriftlig og mundtlig information, og jeg ved nok om studiets formål, metode, fordele og ulemper til at sige ja til at deltage.

Jeg ved, at det er frivilligt at deltage, og at jeg altid kan trække mit samtykke tilbage uden at miste mine nuværende eller fremtidige rettigheder til behandling.

Jeg giver samtykke til at deltage i forskningsprojektet, samt til at projektansvarlige kan tilgå mine journaloplysninger. Jeg har fået en kopi af dette samtykkeark samt en kopi af den skriftlige information om projektet til eget brug.

Forsøgspersonens navn:_____

Dato: _____Underskrift: _____

Ønsker du at blive informeret om forskningsprojektets resultat?

Ja _____ (sæt x) Nej _____ (sæt x)

Erklæring fra den, der afgiver information:

Jeg erklærer, at forsøgspersonen har modtaget mundtlig og skriftlig information om forsøget.

Efter min overbevisning er der givet tilstrækkelig information, til at der kan træffes beslutning om deltagelse i forsøget.

Navnet på den, der har afgivet information:

Dato: _____Underskrift: _____

Dette studie er godkendt af Region Hovedstadens datatilsyn (P-2019-219), Region Hovedstadens Videnskabsetiske Komite (H-20046592) og Lægemiddelstyrelsen (2020070016)

EudraCT: 2020-002459-40
The BREAST-AB Trial
Samtykkeerklæring version 2.0

FORSØGSDELTAGERS VERSION: rives af og gives til forsøgsdeltager

Informeret samtykke til deltagelse i et sundhedsvidenskabeligt forskningsprojekt.

Titel: Forebyggende behandling med lokal antibiotika af brystimplantater til brystrekonstruktion

Originaltitel: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Erklæring fra forsøgspersonen:

Jeg har fået skriftlig og mundtlig information, og jeg ved nok om studiets formål, metode, fordele og ulemper til at sige ja til at deltage.

Jeg ved, at det er frivilligt at deltage, og at jeg altid kan trække mit samtykke tilbage uden at miste mine nuværende eller fremtidige rettigheder til behandling.

Jeg giver samtykke til at deltage i forskningsprojektet, samt til at projektansvarlige kan tilgå mine journaloplysninger. Jeg har fået en kopi af dette samtykkeark samt en kopi af den skriftlige information om projektet til eget brug.

Forsøgspersonens navn:_____

Dato: _____Underskrift: _____

Ønsker du at blive informeret om forskningsprojektets resultat?

Ja _____ (sæt x) Nej _____ (sæt x)

Erklæring fra den, der afgiver information:

Jeg erklærer, at forsøgspersonen har modtaget mundtlig og skriftlig information om forsøget.

Efter min overbevisning er der givet tilstrækkelig information, til at der kan træffes beslutning om deltagelse i forsøget.

Navnet på den, der har afgivet information:

Dato: _____Underskrift: _____

Dette studie er godkendt af Region Hovedstadens datatilsyn (P-2019-219), Region Hovedstadens Videnskabsetiske Komite (H-20046592) og Lægemiddelstyrelsen (2020070016)

EudraCT: 2020-002459-40
The BREAST-AB Trial
Consent form version 2.0

INVESTIGATORS VERSION: Is to be signed and collected with written consent

Informed consent for participation in a health science research project

Titel: Prophylactic treatment with locally applied antibiotics on breast implants for breast reconstruction

Original titel: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Declaration from the trial participant:

I have received written and oral information about the trial, and I have obtained sufficient knowledge regarding the trial's purpose and methods including any advantages and disadvantages related to participation in the trial.

I am aware that it is voluntary to participate, and that I can withdraw my consent at any time during the trial without losing my current or future rights to treatment.

I hereby give consent to participate in the trial and that those responsible for the trial can obtain information from my medical record. I have received a copy of this consent form along with a copy of the participant information regarding the trial.

Name of the participant: _____

Date: _____ Signature: _____

Do you wish to be informed about the results of the trial?

Yes _____ No _____

Declaration from the investigator providing the oral information:

I hereby declare that the trial participant has received oral and written information regarding the trial.

In my opinion, sufficient information has been provided to enable a decision on participation in trial.

The name of the investigator who provided the information:

Date: _____ Signature: _____

This study is approved by the Danish Data Protection Agency (P-2019-219), The Ethics Committee of the Capital Region (H-20046592) and the Danish Medicines Agency (2020070016)

EudraCT: 2020-002459-40
The BREAST-AB Trial
Consent form version 2.0

PARTICIPANTS VERSION: handed over to the participant

Informed consent for participation in a health science research project

Titel: Prophylactic treatment with locally applied antibiotics on breast implants for breast reconstruction

Original titel: Prophylactic treatment of breast implants with a solution of gentamicin, vancomycin and cefazolin antibiotics for women undergoing breast reconstructive surgery: a randomized controlled trial (The BREAST-AB trial)

Declaration from the trial participant:

I have received written and oral information about the trial, and I have obtained sufficient knowledge regarding the trial's purpose and methods including any advantages and disadvantages related to participation in the trial.

I am aware that it is voluntary to participate, and that I can withdraw my consent at any time during the trial without losing my current or future rights to treatment.

I hereby give consent to participate in the trial and that those responsible for the trial can obtain information from my medical record. I have received a copy of this consent form along with a copy of the participant information regarding the trial.

Name of the participant: _____

Date: _____ Signature: _____

Do you wish to be informed about the results of the trial?

Yes _____ No _____

Declaration from the investigator providing the oral information:

I hereby declare that the trial participant has received oral and written information regarding the trial.

In my opinion, sufficient information has been provided to enable a decision on participation in trial.

The name of the investigator who provided the information:

Date: _____ Signature: _____

This study is approved by the Danish Data Protection Agency (P-2019-219), The Ethics Committee of the Capital Region (H-20046592) and the Danish Medicines Agency (2020070016)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page No
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	2
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
	2b	All items from the World Health Organization Trial Registration Data Set	4-18
Protocol version	3	Date and version identifier	Full protocol in supplementary material (SM)
Funding	4	Sources and types of financial, material, and other support	18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	3, 18
	5b	Name and contact information for the trial sponsor	3, 18
	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	18
	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)	18
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	6

	6b	Explanation for choice of comparators	Full protocol in SM
Objectives	7	Specific objectives or hypotheses	6, 9-10
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, noninferiority, exploratory)	7-8, 10-11
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7, full protocol in SM
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)	7-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	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)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	NA
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7-8
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	9-11
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)	See Figure 2
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	11

Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	NA
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	8-9
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	Full protocol in SM
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Full protocol in SM
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	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. Reference to where data collection forms can be found, if not in the protocol	9-11, full protocol in SM
	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	Full protocol in SM
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	Full protocol in SM

Statistical methods	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	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	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)	Full protocol in SM
Methods: Monitoring			
Data monitoring	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	11, Full protocol in SM
	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	Full protocol in SM
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12, Full protocol in SM
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Full protocol in SM
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4, 12
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)	4, full protocol in SM
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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	Full protocol in SM

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
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	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	Full protocol in SM
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Example of patient consent form in SM
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	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.