Local injection of methylprednisolonacetat to prevent seroma formation after mastectomy

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ABSTRACT

INTRODUCTION: This study served the following three purposes: To evaluate the prophylactic effect against seroma of a single dose of steroid in the mastectomy cavity, to evaluate the thesis that there is a connection between subclinical bacterial colonization and seroma formation and to evaluate if a simple urine stix test can detect postmastectomy infection.

MATERIAL AND METHODS: This was a double-blinded and randomized study of injection of methylprednisolonacetate versus saline in the mastectomy cavity at the time of drain removal. A total of 160 females were enrolled after mastectomy. The study parameters were as follows: seroma volume, number of seroma punctures, frequency of clinical infections, degree and type of subclinical colonization, complications and evaluation of the microbiological results of the stix test with automatically read glucose, ketones, blood, pH, protein, nitrite and leucocytes. The degree of inflammation was monitored by measurement of 15 cytokines in each sample of seroma fluid. The study was initiated in August 2010 and is expected to run for three years.

DISCUSSION: Some reports have concluded that seroma formation forms part of postsurgical inflammation. Steroids are effective against inflammation and accumulation of fluid at the surgical site after several types of surgery and have also proved valuable in the treatment of seroma formation. In the present study, the prophylactic effect of steroids on seroma formation is investigated.

CONCLUSION: As the incidence of post-mastectomy seroma formation is 80%, there is a need for improvement in the prophylaxis and treatment of this condition.

FUNDING: not relevant.

TRIAL REGISTRATION: Medicines Agency The EudraCT number 2009-016650-40 has been issued for your Sponsor's Protocol Code Number 23837. Data protection agency J.no. F.750.75-2. The study is perfomed in collaboration with the GCP Unit, capital Region, Bispebjerg Hospital under the EudraCT number: 2009-016650-40.

Seroma formation is a common sequelae after mastectomy and axillary dissection. The incidence ranges from 30% to 92% according to definitions. A seroma is a "necessary evil"; it occurs unpredictably in a predictable number of patients [1].

A seroma was traditionally considered an accumulation of lymphatic fluid [2]. However, it has been demonstrated that a seroma is more than a mere accumulation of serum, probably an inflammatory response forming part of the initial phase of wound healing [3]. McCaul et al [4] concluded that fluid collection after breast cancer surgery and axillary clearance reflects the exudative phase of wound repair. Schulze et al [5] demonstrated that it was possible to inhibit the inflammatory response with a single preoperative infusion of high-dose steroid in patients undergoing open resection of the colon. Recently, impressive achievements in seroma treatment were described by Taghizadeh et al [6]. Patients who underwent latissimus dorsi reconstruction after mastectomy for breast cancer were randomized to either triamcinolone or saline in the cavity at the initial seroma puncture. The authors observed a statistically significant reduction in the number of punctures, total seroma volume and the duration of seroma production. The treatment was well tolerated with no increase in complications or cases of infection. Based on these findings, the aim of the present project was to assess whether methylprednisolonacetate administered in the mastectomy cavity at the time of drain removal prevents or reduces seroma formation. Furthermore, we aimed to study the significance of subclinical microbiology in seromas and to evaluate the diagnostic significance of a simple dip stix test with respect to infections.

THE STUDY

The study comprised three projects:

Project A (seroma prophylaxis)

This project aimed to assess whether instillation of methylprednisolonacetate (Depo-Medrol) 80 mg into the cavity at drain removal after mastectomy (**Table 1**) acts prophylactically against seroma formation.

Project B (microbiology)

This project was designed to determine if bacterial colonization plays a role in seroma formation.

Project C (dip stix test)

This project aimed to establish whether leukocyte ester-

PROTOCOL ARTICLE

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Dan Med J 2012;59(9):A4482

TABLE 1

Inclusion and exclusion criteria.

Inclusion criteria
Age > 18 years
Female
Signed informed consent
Primary breast cancer or ductal carcinoma in situ
Mastectomy with sentinel node biopsy
Mastectomy with/without sentinel node biopsy and full axillary dissection
Excl usion criteria
Previous axillary surgery (made within previous < 4 months)
Recent (< 1 month) treatment with systemic steroids
Allergy to trial drug ingredients
Pregnancy
Inability to understand Danish
Evidence of other relevant medical conditions judged to be inconsistent with participation

TABLE 2

Summary for the reporting of adverse events in clinical trials for non-commercial sponsors.

Adverse reactions/events	The final report of study results to medicines agency
Awaited, and serious suspected adverse reactions	See the list submitted annually including the report on the subjects' safety
Unexpected, serious suspected adverse reactions	Immediately reported by the sponsor. (Serious adverse events /incidents that are deemed not to have a causal relationship with the drug and which are expected, are not subject to this reporting)

TABLE 3

Handling and measurement of cytokines in seroma fluid.

The aspirated seroma fluid is centrifuged at room temperature at 1,500 g for 3 minutes as soon as possible after puncture
The transferred supernatant is placed in two1.5 ml cryotubes, labelled with patient identity and full date (DDMMYY) and stored locally at –20 °C until shipment to the Biobank at Department of Clinical Biochemistry, Gentofte Hospital, Copenhagen for analysis
Quantitative multiplex suspension bead array measurement of interleukin-1beta, interleukin-2, interleukin-4, interleukin-5, interleukin-6, interleukin-10, interleukin-12 (p70), interleukin-13, interferon-gamma, tumour necrosis factor -alpha (Bio-Plex) is analyzed in batch with BioRad, and Bioplex 200 instrument
Samples are destroyed after analysis. The samples will be stored for a maximum of five years

ase and nitrite strip tests may be used to diagnose infection in seroma fluid in women who have undergone mastectomy and axillary dissection.

Inclusion and exclusion

Inclusion and exclusion criteria are described in Table 1. The first patient was enrolled in August 2010. The study is expected to conclude by the end of 2012.

Design

This is a randomized double-blinded study with 40 patients in each group. Paired randomization was performed from random numbers generated from Excel. Codes are kept in sealed envelopes until use.

Calculation of sample size and statistics

The sample size based on project A was calculated for an unmatched case-control study with a continuity correction using the methods of Kelsey, Fleiss and Fleiss [7].

The necessary sample size with a 95% two-sided confidence level and a power of 90% was calculated to 41-47 in each group assuming a 50% reduction in seroma production. Crude associations between categorical variables will be examined by using the χ^2 test. Multiple linear regression models will be used to adjust for potential confounding and risk factors. Continuous variables will be examined by analysis of variance. p values of less than 0.05 will be considered significant.

Ethics

The study will be conducted according to the Helsinki II Declaration. Patients will receive both oral and written information about the study. At our department, approx. 80% of the patients who undergo mastectomy form seromas. Consequently, 10% of the enrolled patients will be receiving redundant medication. It is not possible to identify those 10% in advance, and it is not deemed unethical to implement this project. As noted under side-effects, participation in this study carries no significant risk to patients. Treated patients might benefit from fewer consultations owing to fewer seroma punctures.

Adverse reactions/events/risks

The practical procedure of glucocorticoid/saline administration in the mastectomy cavity is without patient discomfort. A single dose of steroid is not expected to induce either adverse events (AE) or serious adverse events (SAE). According to the Summary of Product Characteristics, Depo-Medrol rarely causes significant systemic side effects. The common side-effects are oedema, adrenal insufficiency, manifestation of latent diabetes mellitus, exacerbation of diabetes, electrolyte imbalance, osteoporosis, headaches, mental disorders and skin atrophy. Theoretically, local steroid administration may increase the risk of wound infection, but breast surgery has a low rate of bacterial contamination, and the literature does not support an increased infection risk associated with steroids. This corresponds to the clinical experience recorded in two reports [6, 8]. A summary of adverse events reported in clinical trials for non-commercial sponsors is presented in **Table 2**.

Data

Study parameters comprise daily seroma production

volume, number of seroma punctures performed by experienced nurses in out-patient clinic (when seroma volume clinically exceeds 50 ml), microbiology in seroma fluid by routine cultivation, microscopy and determination of sensitivity. Microbial assays are simultaneously performed in more sensitive blood culture flasks. At each seroma emptying, a leukocyte esterase and a nitrite dip stix test is read on a Clinitek Status Analyzer (Siemens) for glucose, ketones, blood, pH, protein, nitrite and leucocytes, and 2 ml of seroma fluid is stored for cytokine measurements.

Each participant has a clinical report form (CRF). All study data are immediately recorded in the CRF. Subsequently, data are entered into the FileMaker Pro application and double-checked. When the study period concludes, data will be moved to a SPSS statistical package (IBM) and processed.

Measurement of cytokines

Please see **Table 3** for details of cytokine measurement.

Statistics

Non-parametric tests will be performed. The level of significance is 5%.

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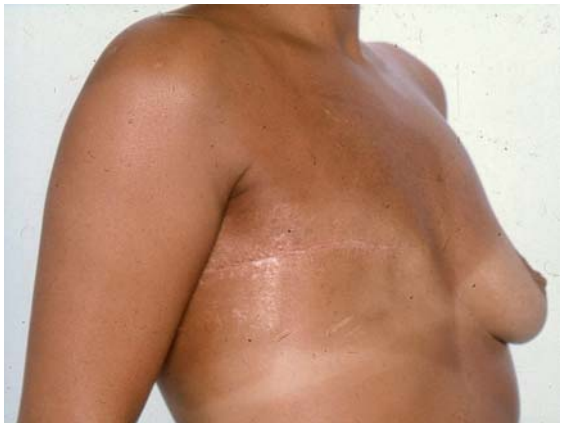
DISCUSSION

A seroma survey [9] failed to identify any significant independent risk factors for seroma formation. Obesity, extended radical mastectomy and a large drainage volume during the first three postoperative days were associated with an increased risk, although these findings were not statistically significant. None of the following could be identified as risk factors: duration of drainage, hormone receptor status, immobilization of the shoulder, intensity of vacuum drainage, nodal status or lymph node metastases, number of lymph nodes removed, number of drains, previous biopsy, removal of drain(s) on day five versus time of minimal drain output, type of drainage, use of fibrin sealant. Other authors have found that obesity, age, hypertension, and the use of electrosurgery predispose to seroma formation [10,11]. There is evidence that the sentinel node technique reduces seroma formation. An aethiologic role of subclinical infections has not been discussed in the literature. In patients with persistent seroma, the fluid is emptied by puncture at an outpatient visit. With each puncture, the risk of complicating infection presumably increases.

The mechanism behind the formation of a seroma is not known in detail. The immunogloublin G, granulocyte and lymphocyte counts in the mastectomy cavity have been compared in patients with and without seroma formation [3]. The levels of immunoglobulin G, leucocytes and granulocytes were higher immediately postoperatively and then declined steadily, whereas the opposite pattern was found for the lymphocyte count which increased steadily over time. It was concluded that a seroma was not just an accumulation of serum, but probably formed part of the postoperative inflammatory response involved in wound healing.

Furthermore, the presence of proteinases, proteinase inhibitors and cytokines (tissue plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), urokinase receptor (uPAR), plasminogen activator inhibitor (PAI)-1 and 2, interleukin 6 (IL-6), and interleukin (IL) 1β) in seroma fluid was demonstrated [12]. Steroids inhibit the inflammatory response through inhibition of cytokine function [5, 13]. Several studies of head and neck surgery have shown that a single dose of 125 mg of methylprednisolone reduced the oedema at the site of surgery [14]. An increased complication rate after surgery in patients treated with a single dose of glucocorticoid has not been demonstrated. In a controlled pilot study, it was tested whether a single dose of glucocorticoid (methylprednisolonsuccinate) given intravenously 1.5 hours preoperatively was effective against seroma formation after mastectomy and axillary dissection. The drainage volume during the first two postoperative days, total seroma volume during days 1-5 and the number of seroma punctures were reduced, but not significantly. The number of seroma punctures and the seroma volume was half that of the control group during the first 6-9 days postoperatively. There were no differences in wound healing time or rate of infectious complications between the groups [8].

The temporary immunosuppression induced by preoperative glucocorticoid infusion [14] may, theoretically,



Mastectomy scar.

be a risk factor in this study. In general, the same factors that stimulate wound healing also stimulate malignant cell growth. Inflammation stimulates the production of cytokines, as do tumour cells. Postoperative studies have demonstrated activity of proteinase, proteinase inhibitors, cytokines and acute-phase reactants in serum and seroma fluid [15,16]. It is estimated that steroids probably do not stimulate, but may have an inhibitory effect on the growth of unrecognized micro-metastases left by cancer surgery.

After mastectomy, it is often difficult to determine whether there is an infection in the cavity. There is a need to establish a simple and rapid method of detecting subclinical infections which may only appear with elevated leukocytes in seroma fluid. It is our hope that the leukocyte esterase and nitrite urine dip stix test will comply with these requirements. Leukocyte esterase and nitrite strip tests have been developed for detection of urinary tract infections. Studies have shown that they can also be used to detect leukocytosis in other liquids, such as peritoneal fluid, with a high sensitivity and specificity [17]. To date, no studies have been performed to examine the validity of such tests performed on seroma fluid. Similarly, it is unknown whether the presence of leukocytosis in seroma fluid is an expression of infection.

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ACCEPTED: 30 May 2012

CONFLICTS OF INTEREST: Disclosure forms provided by the authors are available with the full text of this article at www.danmedj.dk.

THE RESEARCH ETHICS COMMITTEES, CAPITAL REGION: H-4-2009-137

MEDICINES AGENCY: The EudraCT number 2009-016650-40 has been issued for your Sponsor's Protocol Code Number 23837.

DATA PROTECTION AGENCY: J.no. F.750.75-2. The study is performed in collaboration with the GCP Unit, Capital Region, Bispebjerg Hospital under the EudraCT number: 2009-016650-40.

LITTERATURE

1. Gonzalez EA, Saltzstein EC, Riedner CS et al. Seroma formation following breast cancer surgery. Breast J 2003;9:385-8.
2. Kuroi K, Shimozuma K, Taguchi T et al. Pathophysiology of seroma in breast cancer. Breast Cancer 2005;12:288-93.
3. Watt-Boolsen S, Nielsen VB, Jensen J et al. Postmastectomy seroma. A study of the nature and origin ofseroma after mastectomy. Dan Med Bull 1989;36:487-9.
4. McCaul JA, Aslaam A, Spooner RJ et al. Aetiology of seroma formation in patients undergoing surgery for breast cancer. Breast 2000;9:144-8.
5. Schulze S, Andersen J, Overgaard H et al. Effect of prednisolone on the systemic response and wound healing after colonic surgery. Arch Surg 1997;132:129-35.
6. Taghizadeh R, Shoaib T, Hart AM et al. Triamcinolone reduces seroma re-accumulation in the extended latissimus dorsi donor site. J Plast Reconstr Aesthet Surg 2008;61:636-42.
7. www.openepi.com/menu/openEpiMenu.htm
8. Okholm M, Axelsson CK. No effect of steroids on seroma formation after mastectomy. Dan Med Bull 2011;58:A4241.
9. Kuroi K, Shimozuma K, Taguchi T et al. Evidence-based risk factors for seroma formation in breast surgery. Jpn J Clin Oncol 2006;36:197-206.
10. Kumar S, Lal B, Misra MC. Post-mastectomy seroma: a new look into the aetiology of an old problem. J R Coll Surg Edinb 1995;40:292-4.
11. Porter KA, O'Connor S, Rimm E et al. Electrocautery as a factor in seroma formation following mastectomy. Am J Surg 1998;176:8-11.
12. Baker EA, Leaper DJ: Proteinases, their inhibitors, and cytokine profiles in acute wound fluid. Wound Repair Regen 2000;8:392-8.
13. Doolin EJ, Tsuno K, Strande LF et al. Pharmacologic inhibition of collagen in an experimental model of subglottic stenosis. Ann Otol Rhinol Laryngol 1998;107:275-9.
14. Holte K, Kehlet H. Perioperative single-dose glucocorticoid administration: pathophysiologic effects and clinical implications. J Am Coll Surg 2002;195:694-712.
15. Mettler L, Salmassi A, Heyer M et al. Perioperative levels of interleukin-1beta and interleukin-6 in women with breast cancer. Clin Exp Obstet Gynecol 2004;31:20-2.
16. Khan AL, Larsen F, Heys SD et al. Peri-operative acute phase response and cytokine release in women with breast cancer: modulation by polyadenylic-polyuridylic acid. Eur J Surg Oncol 1999;25:574-9.
17. Torun S, Dolar E, Yilmaz Y et al. Evaluation of leukocyte esterase and nitrite strip tests to detect spontaneous bacterial peritonitis in cirrhotic patients. World J Gastroenterol 2007;13:6027-30.