Multicentre, randomised, open-label, non-inferiority trial comparing the effectiveness and safety of ductal lavage versus oral corticosteroids for idiopathic granulomatous mastitis: a study protocol

Tingting Hu,1 Shunrong Li,1 Heng Huang,2 Hui Huang,3 Luyuan Tan,1 Yanbo Chen,4 Heran Deng,1 Jiannan Wu,1 Liling Zhu,1 Jian Zhang,5 Fengxi Su,1 Kai Chen •1

ABSTRACT

Introduction The ideal treatment for idiopathic granulomatous mastitis (IGM) remains unclear. In a prospective, single-centre, pilot study, we reported that ductal lavage treatment for non-lactational mastitis patients had a 1-year clinical complete response (cCR) rate of >90%, without any significant adverse events. Thus, in this multicentre, randomised, open-label, non-inferiority trial, we will aim to compare the effectiveness and safety of ductal lavage vs oral corticosteroids as the first-line treatment for patients with IGM.

Methods and analysis The trial will be conducted at the Breast Tumor Center of Sun Yat-sen Memorial Hospital in China and at least at one participating regional centre. We plan to recruit 140 eligible IGM patients who will be randomised into the ductal lavage group or oral corticosteroid group with a 1:1 ratio. The patients in the oral corticosteroid group will receive meprednisone or prednisone for 6 months. The patients in the ductal lavage group will receive ductal lavage and breast massage, as previously reported. All the participants will be followed up at the clinic for 1 year post randomisation.

The primary endpoint of this trial will be the 1-year cCR rate, and the secondary endpoints will include the time to cCR, treatment failure rate, relapse rate and protocol compliance rate. The trial was designed to determine whether ductal lavage is non-inferior to oral corticosteroids (1-year cCR rate assumed to be 90%), with a non-inferiority margin of 15%.

Ethics and dissemination The ethics committee of Sun Yat-sen Memorial Hospital at Sun Yat-sen University approved the study (2018-Lun-Shen-Yan-No. 30). The results of the trial will be communicated to the participating primary care practices, published in international journals and presented at international clinical and scientific conferences.

Trial registration number ClinicalTrials.gov Registry (NCT03724903); Pre-results.

BACKGROUND

Non-lactational mastitis refers to a group of benign breast diseases. Among these diseases, idiopathic granulomatous mastitis (IGM) is the most commonly reported benign breast disease in clinical practice and the current literature.1 Several treatment approaches have been proposed for IGM patients, such as oral steroids,2–4 surgery,3 immunosuppressive therapy5 6 and observation alone.7 However, most of these studies were retrospective, single-centre and non-randomised studies, rendering their conclusions inapplicable to clinical practice. There is no international consensus and there are no guidelines on the standard treatment of IGM patients. A Chinese experts’ consensus8 recommended oral corticosteroids with or without surgery for IGM patients. However, that supporting evidence for this recommendation was a single-centre, single-arm study that included only 33 patients.4 Hence, a confirmatory study is needed to guide clinical practice. We proposed that the infusion of antibiotics and corticosteroids by ductal lavage is effective for IGM patients and leads to fewer adverse
effects. In our previous study,1 we showed that patients treated with only ductal lavage had a >90% clinical complete response (cCR) rate, without the need for oral corticosteroid treatment. No significant adverse events related to the procedure occurred. To further investigate the effectiveness and safety of ductal lavage as a treatment option for IGM patients, we initiated a multicentre, randomised, open-label, non-inferiority trial to compare ductal lavage vs oral corticosteroids as the first-line treatment of IGM patients. We hypothesised that compared with oral corticosteroids, ductal lavage can provide non-inferior therapeutic effects with significantly fewer adverse events. This trial will provide high-level evidence on the treatment options for patients with IGM.

OBJECTIVE
The objective was to compare the clinical effectiveness and adverse events of ductal lavage vs oral corticosteroids in a multicentre, randomised, open-label, non-inferiority trial. We aimed to provide a high-quality level of evidence to guide clinical practice.

METHODS AND ANALYSIS
Overall study design
This is a multicentre, randomised, open-label, non-inferiority trial that will be conducted in one initiating academic hospital and at least at one participating regional centre in China. The Breast Tumor Center of Sun Yat-sen Memorial Hospital at Sun Yat-sen University will be the initiating centre. Study centres will be eligible to participate in the study if ductal lavage treatment and the relevant protocols can be conducted in the centre. The protocol of this trial was developed according to the Standard Protocol Items: Recommendations for Interventional Trials guidelines.9

Recruitment
A total of 140 patients will be recruited from the participating centres. Patient enrollment started on 29 March 2019, and is expected to be completed on 30 May 2021. Potentially eligible patients will be referred to the site principle investigator for a consultation on the study details. The patients will be informed about the design and methods of the study and the benefits and potential risks of participating in this trial. A screening procedure will be performed if the patients agree to participate, and they will sign an informed consent form.

Screening procedure and inclusion and exclusion criteria
The patients’ medical history, physical examination findings, lab test results and imaging findings will be collected to identify eligible patients.

Patients are eligible to participate in the study if they
► are female and between 18 and 65 years old.
► signed an informed consent form.

► were clinically diagnosed with non-lactating mastitis, defined as mastitis occurring more than 1 month after the cessation of lactation.
► were clinically and pathologically diagnosed with IGM.
► never underwent surgical treatments or oral corticosteroid therapy for mastitis after the cessation of lactation, excluding core needle biopsy.
► were in good health and could undergo ductal lavage, as assessed by physicians.
► had an M-score ≥2.

Exclusion criteria
Patients will be excluded if they
► have a grade III inverted nipple.10
► have lactational mastitis.
► have bilateral IGM.
► were clinically diagnosed with periductal mastitis.
► were pathologically diagnosed with breast carcinoma.
► are pregnant.
► have evidence suggesting a possible diagnosis of systemic lupus erythematosus (SLE), rheumatic disorders or other systemic autoimmune diseases.
► have evidence suggesting a possible diagnosis of tuberculosis.
► have evidence suggesting a possible diagnosis of fungal infection of the breast.
► have a history of breast trauma.
► previously consumed oral corticosteroids or underwent antituberculosis treatment.
► have imaging findings indicating that there are foreign objects retained in the breast.
► have sepsis or severe inflammation caused by IGM, for which surgery is likely required.
► have inappropriate cardiac, pulmonary, liver, renal and coagulation function, leading clinicians to suggest the patient is not suitable for participation in this study.

Definition of the M-score
The M-score was defined as the sum of the following scores:

Mass score
► 0 for the absence of a mass by palpation.
► 1 for mass ≤3 cm by palpation.
► 2 for mass >3 cm by palpation.

Erythema score
► 0 and 2 for the absence and presence of skin erythema, respectively.

Fistula score
► 0 and 2 for the absence and presence of fistula, respectively.

Pain score
► 0 for Visual Analogue Score (VAS) 0–2.
► 1 for VAS 3–5.
► 2 for VAS 6–10.

Quality-of-life (QoL) score
► 0 for the absence of effects on QoL.
► 1 for mild effects on QoL, where the patient does not require medical assistance.
► 2 for serious effects on QoL, where the patient requires medical assistance.

The total M-score ranges between 0 and 10 and serves as a quantitative and objective measurement of the severity of the symptoms.

Randomisation
The information of eligible patients will be uploaded to the REDcap system, which will be based in Sun Yat-sen Memorial Hospital at Sun Yat-sen University.11 We will use the randomised module embedded in the REDcap system, which was based on a list generated a priori, to randomise the participants into the ductal lavage group or oral corticosteroid group with a 1:1 ratio. The randomisation will be stratified by the M-score (≥5 or <5) using a randomly generated block size. The allocation will not be blinded to the patients or the researchers.

Interventions
The patients undergoing oral corticosteroid therapy will receive meprednisone or prednisone 20–40mg four times a day for 2 weeks. Then, the dose will be decreased by 5mg every week to a final dosage of 20mg four times a day. The whole treatment will last for 6 months. The patients undergoing ductal lavage therapy will undergo ductal lavage and breast massage every other day for 2 weeks, as we previously reported.3 We will use lidocaine (1%) for local anaesthesia around the nipple. A lacrimal probe will be used to identify four to five openings in the lactiferous ducts on the nipple, and an infusion cannula (21–23 G) will be inserted. A total of 25mL of irrigation solution (5mL of 2% lidocaine, 40mg of triamcinolone acetonide, 20mL of 0.9% saline and 1.0g of ceftriaxone) will be pumped into the ducts over 20–25min. The patients will return to the clinic the next day for breast massage, and this process will be repeated for 2 weeks. We will continue to follow-up the patients for 12 months after treatment.

Follow-up plans
The participants will return to the clinic for follow-ups at 1, 2, 6 and 12 months post randomisation.

Endpoints
► The primary endpoint will be the cCR rate, which is defined as the proportion of patients who have an M-score of 1 at ≤1 years after treatment.
► The secondary endpoints will be as follows:
  - The time to cCR, defined as the median time to cCR post randomisation.
  - The treatment failure (TF) rate, defined as the proportion of patients with TF at 1 year post randomisation. The TF status will be defined as follows:
    - If the patient has an M-score ≥6 before randomisation, TF is defined as the M-score remaining at ≥6 at 1 month post randomisation.
    - If the patient has an M-score between 4 and 5 before randomisation, TF is defined as the M-score remaining at ≥4 and never being lower than 4.
    - If the patient has an M-score <4 at baseline but never reaches cCR after randomisation, TF is defined as the M-score being >5 at the follow-up and remaining above 5 for one month.
    - Relapse rate, which is defined as the proportion of patients who have an M-score >4 among the patients who achieved CR.
    - Predefined adverse events and any other unexpected adverse events, which will be recorded and compared between the two arms. The following adverse events constitute predefined events:
      - Ductal lavage treatment, defined as bleeding during the procedure, bleeding after the procedure, nipple necrosis or ischemia and skin allergy or pruritus.
      - Oral corticosteroids, defined as thrombosis, metabolic disorders, cardiovascular events, peptic ulcer (including stomach pain), osteoporosis or compression fracture, eye complications, neuropsychiatric system disorder, moon facies, acnitis and facial hair growth.
      - Protocol compliance rate, the proportion of patients who received the treatment protocol.

Researchers from all participating centres will be trained to perform the ductal lavage and/or oral corticosteroid treatment protocol, as well as administer the M-score assessment. Each participant will be separately assessed by a doctor and a nurse for the outcome assessment. If there are any discrepancies in the results between the two assessors, a discussion with a third doctor will be required. The treatment will be stopped if the patients experience serious adverse effects (≥grade 3 on the The Common Terminology Criteria for Adverse Events(CTCAE) 3.0 scale) or meet the TF standard.

Rescue treatments
If inflammation/symptoms worsen during the study treatment, potential second-line/rescue treatments will be discussed between the physicians and the patient on a case-by-case basis. Crossovers (from ductal lavage to oral steroids and vice versa) are allowed, and treatments other than these methods, including surgery and/or methotrexate, are also allowed.

Data collection
All data will be collected, coded and stored using the REDcap system, which is based in Sun Yat-sen Memorial Hospital at Sun Yat-sen University. The decoding file will be saved to a secure local hospital drive, which will be accessible by only the principal and coordinating researchers.
Sample size and statistical analysis

We will report the continuous and categorical variables as the medians (ranges) and counts (percentages), respectively, for the descriptive analysis. The Mann-Whitney U test and χ²-square test will be used to compare the clinicopathological features between the two groups. The primary and secondary endpoints will be compared by intention-to-treat analysis. We will determine whether there are missing data, and we will use multiple imputation and sensitivity analyses to explore the impact of the missing data.

We hypothesise that the 1-year cCR rate of oral corticosteroid treatment will be 90%. This trial was designed to assess the non-inferiority of ductal lavage compared with oral corticosteroids, and the clinically acceptable non-inferiority margin for the ductal lavage group was defined as a 1-year cCR rate equal to or better than 15%. This non-inferiority margin was decided before the initiation of the study during a consensus meeting with a panel of experts. A loss to follow-up rate of 10% was considered, and it was calculated that 70 participants (total of 140 participants) were needed in each group, with a significance level of α=0.025 and power of β=0.80. If non-inferiority is confirmed and the lower border of the 95% CI is >0, the superiority effects of ductal lavage vs oral steroids can be suggested.

An exploratory analysis will be performed to investigate the potential interaction between treatment benefits and the baseline clinicopathological factors, for example, M-score (≥5 or <5). Subgroup analysis will be performed for significant interactions. Longitudinal data analysis will be performed using marginal generalised estimating equations for the cCR, as well as the random effects/mixed models for the M-scores. All of the statistical analyses will be conducted using STATA V.13 statistical software (StataCorp, College Station, Texas, USA).

Ethics and dissemination

This study was approved by the ethics committee of Sun Yat-sen Memorial Hospital at Sun Yat-sen University (2018-Lun-Shen-Yan-No.30). All patients will be fully informed of the potential benefits and disadvantages of all available treatments involving ductal lavage and oral corticosteroids. Additionally, the patients will be required to provide written informed consent. The study, named ‘Ductal Lavage Versus Corticosteroids Therapy for Idiopathic Granulomatous Mastitis’, is registered at ClinicalTrials.gov. As required, a summary of the study results will be submitted to ClinicalTrials.gov. Any important protocol modifications (eg, changes to the primary endpoint, outcomes and analyses) will be reported to ClinicalTrials.gov and the ethics committee of Sun Yat-sen Memorial Hospital at Sun Yat-sen University. The results of the trial will be communicated to the participating primary care practices, published in international journals and presented at international clinical and scientific conferences.

Patient and public involvement

No patients were involved.

DISCUSSION

Oral corticosteroid therapy as the control condition

Since the aetiology of IGM is still unclear, there is no international consensus on the best first-line treatment for IGM patients. We were the first to report that ductal lavage, a novel approach, is feasible and safe for treating IGM patients in a single-arm, phase II trial. Currently, oral steroids²⁻⁴ ¹² and/or observation alone⁷ ¹³ are the most commonly used approaches worldwide.¹⁴ In 2016, a Chinese group of experts⁸ recommended the use of oral corticosteroids (20–40 mg four times per day) with or without surgery as the first-line treatment for IGM. However, the evidence was based on a single-centre, retrospective study that included only 33 patients. In our daily clinical practice, we routinely prescribe oral corticosteroids (20–40 mg four times per day) for IGM patients, and the efficacy and safety are acceptable. Thus, we conducted this randomised trial to directly compare the efficacy and adverse events of ductal lavage and oral corticosteroids (20–40 mg four times per day). Although observation alone is an option for the treatment of IGM,⁷ ¹³ it is not included as a control condition in this trial because we, including the ethics committee, do not consider it to be ethical to only observe patients without offering any treatment. Our previous phase II trial showed that the M-scores, as well as the VAS scores, significantly decreased at 1 week after ductal lavage treatment,¹ which is unlikely to occur if we use the ‘observation alone’ strategy without any treatments. Furthermore, 65.6% (21/32) of the patients achieved cCR in ≤6 months¹, and the time to cCR appeared to be shorter than that for observation alone.⁷ ¹³

Safety and rationale for the use of ductal lavage for IGM

The aetiology, as well as the pathophysiology of IGM, is still unclear. Several hypotheses have been proposed, such as hypersensitivity to extravasated lactation products, local breast trauma, subclinical infection and autoimmune reactions.¹⁵⁻¹⁷ However, none of these hypotheses have been widely accepted. Therefore, this disease was named IGM. Ductal lavage was first proposed as a method of collecting breast epithelial cells for cytologic analysis. The procedure uses a microcatheter to cannulate ductal orifices on the nipple. Each duct is cannulated and infused with normal saline.¹⁸ The safety and tolerability of this procedure have been demonstrated in multiple studies.¹⁸ ¹⁹ In clinical practice, oral corticosteroids are widely reported as an effective treatment for IGM. Therefore, we speculate that a small change in the drug delivery approach from oral to ductal lavage might yield the same level of efficacy but fewer adverse events.

Selection of ducts for lavage

Whether it is necessary to cannulate all of the ducts or just the duct that leads to the lesion is an important question.
The study conducted by Stearns *et al* showed that the drugs were distributed widely throughout the entire breast, regardless of which duct was cannulated. In addition, the number of orifices that could be cannulated in clinical practice differed from that of the histological orifices on the nipple. In our study, we will choose four to five orifices randomly, as we consider this method clinically feasible, and it has been proven to be effective in our previous phase II study. We believe that this ductal lavage procedure can be used to deliver the drugs to the target lesions.

**M-score to quantify the severity of symptoms**

IGM is a benign disease, and the need for medical treatment depends on the patients’ symptoms. For pathologically confirmed IGM patients presenting with a small mass without any pain, fistulas, tenderness or erythema of the skin, the IGM lesion might not affect the patients or require medical treatment. Thus, this trial developed an M-score to quantify the severity of symptoms in IGM patients, for whom higher M-scores reflect more severe symptoms. An important application of the M-score is in quantifying the treatment response. In this trial, we defined an M-score ≤1 as cCR, which is objective and easy to replicate in other institutions. To the best of our knowledge, most of the published studies in the literature did not use clear or objective criteria to define cCR. With the M-score, the severity of symptoms (or the need for medical treatment) and the treatment response can be quantified.

**Differential diagnosis between IGM and PDM**

Studies have shown that periductal mastitis (PDM) tends to recur, and the removal of all of the involved ducts and fistula tract in its entirety is widely recommended. In our previous study, we observed that half of the (2/4) PDM patients experienced recurrence after the treatment of IGM. Thus, we will exclude patients with a definitive clinical diagnosis of PDM from this trial. The differential diagnosis between IGM and PDM is based on clinical manifestations, as well as pathology examination findings. The typical clinical manifestations of PDM include a periareolar inflammatory mass and/or an intermittently draining fistula. The IGM mass can be present in any quadrant of the breast, with characteristics including erythema, tenderness, abscess and sinus formation with drainage. Pathologically, IGM is characterised by granulomatous centres around the lobules and ducts, while PDM is characterised by keratinising squamous epithelium to orifices of the nipple ducts. However, it is not always possible to differentially diagnose IGM and PD in clinical practice. The symptoms of the two conditions can overlap. In our study, we only excluded patients with a definite diagnosis of PDM.

**Sample size estimation**

The aim of this study is to determine whether ductal lavage treatment is non-inferior to oral corticosteroid treatment. Our previous meta-analysis showed that the cCR rate was 71.8% (95% CI 67.1% to 76.3%). In our clinical experience, however, the cCR rate might be higher than 71.8%. Thus, we hypothesise that the 1-year cCR rate is 90% in the corticosteroid group. We consider the 15% non-inferiority margin to be appropriate because if the patients are responsive to ductal lavage treatment, oral corticosteroids do not need to be administered. For the patients who do not respond to ductal lavage treatment, oral corticosteroids can still be provided as a second-line treatment, and this approach might not compromise the patients’ clinical outcomes. IGM is different from breast cancer, as delayed treatment may cause significantly worse clinical outcomes in patients with breast cancer.

**CONCLUSION**

The trial has several novel aspects. First, no randomised controlled trials have yielded high-quality evidence to determine the best first-line treatment for patients with IGM. Second, we were the first to propose that ductal lavage treatment is an effective and safe approach for IGM patients. Third, we were the first to propose the use of the M-score to objectively delineate the severity of symptoms in IGM patients, and this score can be used for the objective evaluation of patients’ treatment responses. The aim of this trial is to provide high-quality evidence for the management of IGM patients.

**Trial status**

We began recruiting patients for this trial on 29 March 2019. The study is expected to be completed by 30 May 2022.
the study and revise the manuscript. KC: Helped design the study and the data collection form and write and revise the manuscript.

**Funding** This study is funded by the Sun Yat-sen University Clinical Research 5010 Program (recipient: KC; No. 2018022), the Yat-sen Scholarship of Young Scientist Program of Sun Yat-sen Memorial Hospital at Sun Yat-sen University (recipient: KC; Financial number: 1320217001), grants from the Sun Yat-sen Clinical Research Cultivating Program of Sun Yat-sen Memorial Hospital at Sun Yat-sen University (recipient: KC; SYS-G-201702) and grants from the China Anti-aging Promoting Association (recipient: SL; Financial number: 9100016037). This study is also supported by the National Natural Science Foundation of China (Grant# 81672619), and the grant from Guangdong Science and Technology Department (2017B030314026).

**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not required.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

**ORCID iD**
Kai Chen http://orcid.org/0000-0002-7052-4681

**REFERENCES**

1. Chen K, Zhu L, Hu T, et al. Ductal lavage for patients with Nonlactational mastitis: a single-arm, proof-of-concept trial. *J Surg Res* 2019;235:440–6.

2. Karanlık H, Ozgur I, Simsek S, et al. Can steroids plus surgery become a first-line treatment of idiopathic granulomatous mastitis? *Breast Care* 2014;9:338–42.

3. Yabanoğlu H, Çolakoğlu T, Belli S, et al. A comparative study of conservative versus surgical treatment protocols for 77 patients with idiopathic granulomatous mastitis. *Breast Care* 2010;5:363–9.

4. Eroğen F, Ersoy YE, Akaydin M, et al. Corticosteroid treatment and timing of surgery in idiopathic granulomatous mastitis confusing with breast carcinoma. *Breast Cancer Res Treat* 2010;123:447–52.

5. Akbulut S, Arıkanoğlu Z, Senol A, et al. Is methotrexate an acceptable treatment in the management of idiopathic granulomatous mastitis? *Arch Gynecol Obstet* 2011;284:1189–95.

6. Akbulut S, Yılmaz D, Bakır S. Methotrexate in the management of idiopathic granulomatous mastitis: review of 108 published cases and report of four cases. *Breast J* 2011;17:661–8.

7. Bouton ME, Jayaram L, O’Neill PJ, et al. Management of idiopathic granulomatous mastitis with observation. *Am J Surg* 2015;210:258–62.

8. CNKI. Experts consensus of nonlactational mastitis, 2016. Available: http://www.cnki.com.cn/Article/CJFDOTAL-ZGWK201607014.htm

9. Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200–7.

10. Han S, Hong YG. The inverted nipple: its grading and surgical correction. *Plast Reconstr Surg* 1999;104:389–95.

11. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377–81.

12. Aghajanzadeh M, Hassanzadeh R, Alizadeh Safat S, et al. Granulomatous mastitis: presentations, diagnosis, treatment and outcome in 206 patients from the North of Iran. *Breast* 2015;24:456–60.

13. Hur SM, Cho DH, Lee SK, et al. Experience of treatment of patients with granulomatous lobular mastitis. *J Korean Surg Soc* 2013;85:1–6.

14. Lei X, Chen K, Zhu L, et al. Treatments for idiopathic granulomatous mastitis: systematic review and meta-analysis. *Breastfeed Med* 2017;12:415–21.

15. Al-Khaffaf B, Knox F, Bundred NJ. Idiopathic granulomatous mastitis: a 25-year experience. *J Am Coll Surg* 2008;206:269–73.

16. Sakurai K, Fujisaki S, Enomoto K, et al. Evaluation of follow-up strategies for corticosteroid therapy of idiopathic granulomatous mastitis. *Surg Today* 2011;41:333–7.

17. Yau FM, Macadam SA, Kuusk U, et al. The surgical management of granulomatous mastitis. *Ann Plast Surg* 2010;64:9–16.

18. Dooley WC, Ljung BM, Veronesi U, et al. Ductal lavage for detection of cellular atypia in women at high risk for breast cancer. *J Natl Cancer Inst* 2001;93:1624–32.

19. Evron E, Dooley WC, Umbricht CB, et al. Detection of breast cancer cells in ductal lavage fluid by methylation-specific PCR. *Lancet* 2001;357:1353–6.

20. Stearns V, Mori T, Jacobs LK, et al. Preclinical and clinical evaluation of intraductally administered agents in early breast cancer. *Sci Transl Med* 2011;3:106ra108.

21. Fucking M, Woman J, Collins F, et al. Sabiston textbook of surgery: the biological basis of modern surgical practice. Philadelphia: Saunders, 2007.

22. MTA Mol. Atlas of ultrasound and ductal echography of the breast: the introduction of anatomic intelligence into breast imaging. 1 edn. Hoboken, New Jersey: Wiley-Blackwell, 1995.

23. Flanagan M, Love S, Hwang ES. Status of intraductal therapy for ductal lavage fluid by methylation-specific PCR. *Cancer Inst* 2001;93:1624–32.

24. Dooley WC, Ljung BM, Veronesi U, et al. Ductal lavage for detection of cellular atypia in women at high risk for breast cancer. *J Natl Cancer Inst* 2001;93:1624–32.

25. Vinay Kumar AKA, Aster. Robbins JC. *Cotran pathologic basis of disease*. Amsterdam, Netherlands: Elsevier, 2014.

26. Susan C, Lester DGH. *Diagnostic pathology: breast*. 2 edn. Elsevier, 2016.