**Abstract**

**Introduction.** Breast cancer is the most common cancer in Indonesia with incidence rate 40.3 per 100,000 women and mortality rate 16.6 per 100,000 women. On early stage, the decision for operative procedure (i.e. mastectomy) requires intraoperative frozen section to assess malignancy; which is mostly unavailable in secondary hospitals. The triple diagnostic (TD) test consists of physical examination, breast ultrasonography and fine needle aspiration biopsy is an accurate and simple preoperative diagnostic method that may solve the problem. The study aimed to find out conformance of the triple diagnostic to histopathology findings in those with breast lump where the malignancy was suspected.

**Method.** A study of diagnostic accuracy conducted enrolling subjects with suspected malignant breast lump managed in dr Cipto Mangunkusumo General Hospital (RSCM) and Persahabatan Hospital (RSP) in period of February 2016 to August 2017 who met the criteria: those underwent preoperative triple diagnostic, intraoperative frozen section and histopathology examination. The conformance of TD and frozen section were compared to histopathology findings. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were the focuses of the study.

**Results.** There were 33 subjects enrolled (prevalence of 4.3%), mean age of 49.6 years ± 10.9, were above 40 years (78.8%). Tumor size of 2–5 cm found in 63.6% subjects, and the most histopathology finding was invasive carcinoma (84.8%). Frozen section showed sensitivity of 96.8%, specificity of 100%, PPV of 100%, NPV of 66.7% and accuracy of 97.0%. TD showed sensitivity of 77.4%, specificity of 100%, PPV of 100%, NPV of 22.2% and accuracy of 78.8% (p = 0.016).

**Conclusion.** Triple diagnostic reaches up to 78% accuracy on early stage breast cancer may be used secondary hospital in Indonesia whenever frozen section is unavailable.

**Keywords:** early stage breast cancer, triple diagnostic, frozen section, histopathology

**Introduction**

Breast cancer is the most common cancer with incidence rate 40.3 per 100,000 women or 48,998 new cases per year (meanings that there are six new cases of breast cancer each hour) and a mortality rate as high as 16.6 per 100,000 women or as many as 19,750 women per year.¹ According to Indonesian national health insurance (BPJS), in 2015 total expenses for cancer was 1.318 trillion rupiah with a total of 724,636 cases in Indonesia.² In 2014–2016, there were 335 new breast cancer cases in dr. Cipto Mangunkusumo General Hospital (RSCM),³ and Rachamawati reported (2012) out of 126 there were 112 of cases (88.88%) admitted as advanced stage at RSCM.⁴

The final diagnosis of breast cancer is established with histopathology findings which is set as the gold standard.⁵ Nowadays, the method of diagnostic in RSCM was in accordance with the guideline provide by Association of Indonesian Oncology Surgeons (PERABOD), namely history taking, physical examination, tissue biopsy, ultrasonography or mammography, surgical excision with frozen section that may leading to decide the resection of choice (for instance, mastectomy).⁶ Unfortunately the facility to provide histopathology and frozen section examination is not available in all hospital due to the distribution of pathologist as well as the facilities; thereby influencing the prognosis.⁷ It is in this unavailability, other diagnostic method is needed. An alternative is a simple triple diagnostic test.⁸

Triple diagnostic test introduced in 1970 as a diagnostic method in evaluation of breast lumps. This test consists of three components namely physical examination, ultrasound and/or mammography, and fine needle aspiration biopsy (FNAB).⁹ The combination of the three components is essential: 1) Clinical examination independently showed the specificity, sensitivity, and accuracy >80%; however it is not a stand-alone method as the basis for definitive treatment. 2) Ultrasound and/or mammography may detect a clinically palpable small tumor showed the specificity, sensitivity, and accuracy >70%. However, the limitation of a method is not recommended for women <35 years due to high breast density and the facility is not available in all hospital. 3) Cytology examination of specimen taken by fine needle aspiration is an early detection tools with the specificity, sensitivity, and accuracy >87%.³ Triple diagnostic has been shown to be useful as an accurate diagnostic tool, technically simple, and proven to reduce the total medical expenses as well as morbidity.⁹

Should the diagnosis established preoperatively, then the benefits are: 1) Patients and their family may be given a detailed operative measures that will be carried out which is mutilating in nature, so the
patient is well prepared psychologically; 2) Patients with benign abnormalities do not need to undergo further excessive diagnostic test for staging purpose; and 3) Intra-operative frozen section will be unnecessary and therefore may reduce surgical duration and operational expenses, as well as anesthesia.6

Currently, triple diagnostic has not been used as a diagnostic guide in patients with breast lumps at RSCM and Persahabatan Hospital (RSP). Thus, the accuracy of its predictive value is unknown.

Method

A diagnostic study proceeded on early stage breast cancers managed at RSCM and RSP in period of February 2016 – August 2017 to find out the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of triple diagnostic and frozen section compared to the gold standard (histopathology). Triple test score found from triple diagnostic method were classified to concordant malignant (TTS = 9), suspicion malignant (TTS 4–8) and concordant benign (TTS = 3). In this study, only subjects with TTS 4–8 were enrolled and were subjected to further classified into low margin (TTS 4–6) and high margin (TTS 7–8). Patients who came for the first time or who came to control at surgical oncology clinic in both hospitals within the period was enrolled. A consecutive sampling was used, and data were collected from medical report in both hospitals. Subjects were informed who agreed to participate and signed the consent. Males, those whose loss of follow-up, and those with incomplete data were excluded.

Descriptive analysis addressed for demographic, clinical, and laboratory characteristics were expressed in percentage. Data were subjected to analysis using SPSS ver. 20.0 for Macintosh. Normality test carried out on numerical, and means were used in normal distribution. Analysis carried out using McNemar test for triple diagnostic variables. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of triple diagnostic was determined using the standard formula: sensitivity = TP/(TP + FN); specificity = TN/(TN + FP); positive predictive value = TP/(TP + FP); negative predictive value = TN/(TN + FN); and accuracy = (TP + TN)/(TP + TN + FP + FN), with TP is true positive; TN, true negative; FP, false positive; and FN, false negative.

Results

There were 1,095 cases of breast cancer managed in such a period. However, only 41 subjects referred to the early stage (stage 1–2) breast cancer. Eight out of 41 subjects were excluded as the data incomplete. Of a total subject enrolled, 33 subjects were analyzed, whereas 26 subjects managed at RSCM and 7 subjects at RSP.

The subjects were classified based on the staging and TNM system. Sixteen out of thirty-three subjects were of stage IIB (48.6%) as seen in Table 1.

| Table 1. Distribution of subjects based on TNM staging |
|-------------------------------------------------------|
| Stage | TNM | Total (%). |
| IIA  | T1N0M0 | 3 (9) |
| IB   | T2N0M0 | 14 (42.4) |
| IIA  | T1N1M0 | 0 (0) |
| IIB  | T2N1M0 | 7 (21.3) |
|      | T3N0M0 | 9 (27.3) |

Age distribution of these subjects of the early stage (stage I–II) was 49.6 years ± 10.9, mostly (78.8%) of >40 years. Subjects with malignancy had tumor size measuring 2 to 5 cm (63.6%) and of the most histopathology finding was invasive carcinoma (84.8%) (Table 2).

In the study on physical examination, all subjects were categorized as suspicion malignant (100%). While as study on ultrasound examination, three subjects (9.1%) were benign, fourteen subjects (42.4%) were suspicion malignant, and 16 subjects (48.5%) were malignant. On FNAB study, two subjects (6.1%) were benign, fourteen subjects (42.4%) were suspicion malignant and 17 subjects (51.5%) were malignant. On histopathology study, thirty–one subjects (93.9%) were malignant and 2 subjects (6.1%) were benign.

On the study, all subjects were of suspicion malignant category (TTS 4–8), nine subjects (27.3%) were suspicion malignant of low margin (TTS 4–6) and 24 subjects (72.7%) were suspicion malignant of high margin (TTS 7–8). The frozen section showed three subjects (9.1%) were of benign category, two subjects (6.1%) were of suspicion malignant and 28 subjects (84.8%) were of malignant category (Table 3). In this study, all subjects who were categorized as suspicion malignant in each component of triple diagnostic as well as frozen section were categorized as malignant, therefore two categories namely benign and malignant were created. Hereafter, those data were compared with the histopathology as the gold standard.

Table 3. Triple diagnostic components and frozen section

| Component of investigation | Total | % |
|----------------------------|-------|---|
| Physical examination |       |   |
| Benign | 0 | 0 |
| Suspicion malignant | 33 | 100 |
| Malignant | 0 | 0 |
| Ultrasound |       |   |
| Benign | 3 | 9.1 |
| Suspicion malignant | 14 | 42.4 |
| Malignant | 16 | 48.5 |
| FNAB |       |   |
| Benign | 2 | 6.1 |
| Suspicion malignant | 14 | 42.4 |
| Malignant | 17 | 51.5 |
| Triple diagnostic |       |   |
| Suspicion malignant low margin (TTS 4–6) | 9 | 27.3 |
| Suspicion malignant high margin (TTS 7–8) | 24 | 72.7 |
| Frozen section |       |   |
| Benign | 3 | 9.1 |
| Suspicion malignant | 2 | 6.1 |
| Malignant | 28 | 84.8 |
| Histopathology |       |   |
| Benign | 2 | 6.1 |
| Malignant | 31 | 93.9 |
Comparison of frozen section with histopathology finding

Comparison of frozen section with histopathology finding showed that out of all subjects categorized as malignant, thirty subjects (90.9%) were histopathologically confirmed malignant. Out of 3 subjects categorized as benign, two subjects (6.1%) were histopathologically confirmed benign and 1 subject (3.0%) were histopathologically malignant (Table 4). In this study, McNemar test for conformance of the three triple diagnostic components to frozen section and histopathology showed a p value of 1.00.

Comparison of triple diagnostic with histopathology finding

Comparison of triple diagnostic with histopathology finding showed all 24 subjects categorized as suspicion malignant of high margin (TTS 7–8) were confirmed on histopathologically malignant. Out of nine subjects categorized as suspicion malignant of low margin (TTS 4–6), seven subjects were confirmed histopathologically malignant and only 2 subjects were histopathologically benign (Table 5). McNemar test carried out for conformance showed p value of 0.016.

Table 4. Distribution based on frozen section and histopathology results

| Results | Histopathology          |
|---------|-------------------------|
| Frozen | Malignant | %    | Benign | %    | Total | %    |
| Section |            |      |        |      |       |       |
| Malignant | 30        | 90.9 | 0      | 0    | 30    | 90.9 |
| Benign | 1          | 3.0  | 2      | 6.1  | 3     | 9.1  |
| Total | 31         | 93.9 | 2      | 6.1  | 33    | 100  |

Table 5. Distribution based on triple examination and histopathology results

| Results | Histopathology          |
|---------|-------------------------|
| Triple Test Score (TTS) | Suspicion malignant high margin (TTS 7–8) |
|                      | Malignant | %    | Benign | %    | Total | %    |
| SUSP             | 24       | 72.7 | 0      | 0    | 24    | 72.7 |
| Part   | Malig      | 0      | 0      | 0    | 0      | 0      |
| Total | 31         | 93.9 | 2      | 6.1  | 33    | 100   |

*McNemar Test: p=0.0016

Discussion

This is the first study to investigate the triple diagnostic test conformance to histopathology finding enrolling subjects managed at RSCM and RSP. Even though a total of 1,095 breast cancer cases were managed within period of April 2016 to August 2017, the study only focused on early stage breast cancer with the prevalence of 43.8%. Thus, only 41 subjects were enrolled throughout 16 months of data collection, and out of these only 33 subjects were analyzed. The number of subjects enrolled was found lesser than minimal number of samples required for a diagnostic study as eight subjects did not complete the triple diagnostic test or frozen section examination before histopathological investigation. This is found to be reasonable as both hospital of RSCM and RSP are the top referrals characterized with those in advanced stages.

Out of 33 subjects analyzed, mostly were of 49.6 years and 78.8% were more than 40 years. This finding was in line with study of Kharkwal et al in Asian population who found population of 35–70 years. He found that 60% of breast cancer were diagnosed at the age of 41 to 60 years with average of 50 years. Study of Mu et al on 20–80 years population with early stage breast cancer showed the average age of 45 years. In the United States, breast cancer were mostly found in average of 40–70 years, with tumor size of 2 to 4.9 cm at the first diagnosis. While as in United Kingdom, the first diagnosed is at 60–70 years, with tumor size of 2 to 5 cm. The contributing factors to age different of Asian, American and England have not been established. However, it is considered that BRCA1 and 2 gene variations in different population were responsible.

In the study, 63.6% subjects categorized as suspected malignant measuring 2 to 5 cm tumor size, and 18.4% were invasive carcinoma on histopathological findings. Subject characteristics was in line with previous studies found the first diagnosis is in average of 40–50 years, and more aggressive tumors as invasive ductal carcinoma and invasive lobular carcinoma.

The physical examination showed sensitivity of 100% and specificity of 0% was found conformance to histopathological investigation. Positive predictive value was 93.9% and non negative predictive value found because unable to be calculated. The accuracy of physical examination to histopathological investigation was 93.9%. Studies showed that physical examination is subjective but also objective and referred to investigator dependent. Thus, should never be used independently in the establishment of the malignancy of
breast lesion. However, it’s an essential supporting component of triple diagnostic test should be carried out first.

Sensitivity of ultrasound to histopathological finding was 93.5%, and less specificity of 50%. Positive predictive value was found higher (96.7%) than negative predictive value (33%). The accuracy of ultrasound to histopathological finding was 90.9%. Paramita et al found the accuracy in their study was 70.96% with false positive value of 45.45% and false negative value of 20.16. Since ultrasound is operator dependent, the higher accuracy of ultrasound in the study may correlate to the fact that 78.8% of subjects were carried out by experienced radiologist in breast–division (RSCM) and 31.2% were carried out by experienced radiologist in thoracic subdivision (RSP). Nevertheless, study by Kim et al in Korea found the sensitivity of ultrasound for breast cancer detection compared to histopathological examination was 98.4%.

Hasni et al., found that ultrasound for breast cancer detection has sensitivity of 100%, specificity of 85.7%, and accuracy of 87.5%; all procedures were carried out by the same person, i.e. the resident of radiology training of the third year.

The sensitivity of FNAB to histopathological examination was found higher than its specificity (96.8%) compared to 50%. Positive predictive value was found higher (96.8%) than negative predictive value (50%). The accuracy of FNAB to histopathological investigation was 93.9%. In the study, the procedure of FNAB is not guided by ultrasound, let a lower specificity value found. Study showed that ultrasound-guided FNAB is the most dependable element of TTS with sensitivity of 80–91%, specificity of 79–100%, and accuracy of 87.19. This study finding was in line with Kaufman et al who found the sensitivity, accuracy, positive predictive value, and negative predictive value of FNAB in breast cancer detection was 93%, 95%, 96%, and 94% respectively.20 Katti et al also found high sensitivity of 97.56%, with accuracy of 98.94%, positive predictive value of 97.56%, negative predictive value of 98.18%, and specificity of 100%.21 FNAB is influenced by many factors i.e. site of the lump (either superficial or deep), technique in taking the specimen, staining method, and even the pathologist experience interpretation.22

Analysis of sensitivity and specificity of frozen section to histopathological examination is 96.8% and 100% respectively. The positive predictive value of frozen section was higher than its negative predictive value; i.e. 100% compared to 66.7%. While the accuracy of frozen section compared to histopathological examination was 97.0%. Boughey et al found the sensitivity was 94.7%, with specificity of 100%, positive predictive value of 100%, negative predictive value of 92.8%, thereby may reduce reoperation.23 Accuracy of frozen section is influenced by various factors i.e. slicing technique and technique of specimen collection technique, and interpreter.24 In the study, a high accuracy reflecting appropriate specimen collection, technique and interpretation by experienced pathologist. However, frozen section has limitations, i.e. high cost and need a longer surgical duration, and available pathologist in operating theater. Those components are often not available in Indonesia. According to Indonesian Ministry of Health, a total 610 pathologists were not distributed well in Indonesia.

The samples of this study were categorized in two groups for data analysis i.e. suspected malignant low margin (TTS 4–6, 27.3%) and high margin (TTS 7–8, 72.7%). Study of Mokri et al showed that TTS 4–5 shows the tendency to be benign and TTS6 were frequently malignant, thereby should be treated as malignant lesions with 100% sensitivity.25 Conformance analysis of triple diagnostic test to histopathological examination showed lower sensitivity (77.4%) compared to specificity (100%). While the positive predictive value is much higher than negative predictive value (100% compared to 22%). The accuracy of triple diagnostic test to histopathological examination was 78.8%. High positive predictive value (100%) and satisfactory accuracy value (78.8%) is very important to clinicians for establishing the diagnosis of breast cancer using merely triple diagnostic method.

This study findings are slightly different to study of Novianto et al who found sensitivity of 94%, specificity of 95%, positive predictive value of 96%, negative predictive value of 93%, and accuracy of 94% with the combination of clinical examination, breast ultrasound and cytologic investigation taken by aspiration biopsy on early stage breast cancer.15 The difference is those carried out by Novianto et al were operator dependent physical examination. In RSCM, all the ultrasound and FNAB performed and verified by both radiologists and pathologists, let minimize examination biases. However, physical examinations were carried out by surgical residents or trainees.

Accuracy of triple diagnostic test in this study is lower than frozen section (78.8% compared to 97.0%). However, triple diagnostic test is quite accurate and simple which is useful should frozen-section is not available. This finding is like the study of Kauffmann et al indicating the triple diagnostic test is non-concordant; should it concordant then accuracy may up to 77–99%.20 In addition, frozen section requires intra-operative pathologists who are frequently unavailable in all Indonesian hospital due the distribution. In such a case, triple diagnostic test is the best option to establish the diagnosis, decision making, reduces reoperation, and reduces duration of surgery. Kharkwal et al in India showed this triple diagnostic is an accurate non-invasive diagnostic method should be considered for diagnosis and treatment.

Conclusion

The study found prevalence of early stage breast cancer in RSCM and RSP is 4.3%. The conformance of triple diagnostic test showed sensitivity of 77.44%, specificity of 100%, positive predictive value of 100%, negative predictive value of 22.2%, and accuracy of 78.8%. Frozen section showed sensitivity of 96.8%, specificity of 100%, positive predictive value of 100%, negative predictive value of 66.7%, and accuracy of 97%. Triple diagnostic test may be used as an option of diagnostic method in hospitals where frozen section examination is not available.

Conflict of interest

Author disclose there was no conflict of interest.

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