

ORIGINAL

False–negatives due to poor specimens can be prevented based on the macroscopic findings for breast Vacuum–Assisted Biopsy specimens

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Abstract : Purpose : Ultrasound-guided breast tissue biopsy is an essential technique for diagnosing breast disease, but sample errors reduce its accuracy. This study investigated whether the histopathological results can be inferred from the macroscopic findings for Ultrasound-guided breast Vacuum Assisted Biopsy (VAB) specimens. **Methods:** Biopsy specimens from 101 patients who underwent mammary gland VAB were photographed with a smartphone, and the relationships between the macroscopic findings and the pathological results were examined. **Results :** A significant difference was observed with regard to the presence/absence of turbidity: malignancy was detected in 33/37 (89%) specimens with turbidity and in 2/47 (4%) cases without turbidity ($p < 0.001$). A significant difference was also observed regarding the surface properties: malignancy was detected in 14/70 (19%) smooth specimens and in 24/29 (83%) rough specimens ($p < 0.001$). Also, malignancy was detected in 11/13 (85%) specimens with white spots, and the difference was significant ($p < 0.001$). In addition, the characteristics of intraductal papilloma, fibroadenoma, and mastopathy could be confirmed by macroscopic findings. **Conclusions :** When needle–biopsy of a lesion that is targeted for resection yields macroscopic findings that match the predicted histopathological findings, it can be thought that the biopsy had been properly performed. This means that false–negatives due to poor specimens can be prevented. *J. Med. Invest.* 69: 51-56, February, 2022

Keywords : VAB, skill of the operator, histopathological findings, accuracy, sample errors

INTRODUCTION

Needle biopsy is an essential modality for diagnosis of breast disease (1-5). In recent years, many small lesions have been detected due to mammography-combined screening for breast cancer and improvements in breast ultrasound equipment, and the target lesions of needle biopsy have also become smaller. The success or failure of needle biopsy is often influenced by the skill of the operator, but one of the causes of false-negatives is that the lesion was not properly biopsied (6-8). In particular, in the case of small lesions, it is more likely that biopsy will not be performed properly. Calcified lesions can be confirmed by radiography of biopsy specimens, but there is no confirmation method for lesions without calcification (8-11). On the other hand, if the macroscopic findings of a specimen obtained by needle biopsy match the predicted histopathological findings, it can be judged that the lesion was properly biopsied. However, there are no reports on the macroscopic findings for needle biopsy specimens. This retrospective study investigated whether the histopathological results can be inferred from the macroscopic findings for breast Vacuum Assisted Biopsy (VAB) specimens.

SUBJECTS AND METHODS

Macroscopic study of normal breast VAB specimens

Five mammary glands totally resected at Higashi Tokushima Medical Center were immediately subjected to VAB of a site that was thought to be normal. The obtained VAB specimens were photographed and macroscopically confirmed to be normal. The photographic procedure was as follows. A smartphone (iPhone 7) was equipped with a 3x macro-lens. The photo shooting condition setting on the smartphone is automatic. The camera specs are: focal length 3.99mm, f-number (aperture value) 1.8, resolution 3020×4032 pixels, shooting conditions: shutter speed 1/30~420, ISO sensitivity 20~40. The specimen was illuminated with a commercially available illumination device (MAG-LITE SOLITAIRE LED FLASHLIGHT, 47 LUMENS, MAG INSTRUMENT, INC.; USA) at an angle of about 30 degrees to enable observation with tangential light (Fig. 1). The findings were judged by two doctors (one surgeon and one radiologist) and finalized by discussion.

As a result, it was judged that the normal mammary gland tissue in the VAB specimen was transparent and had a smooth and shiny surface. Therefore, our current study of macroscopic findings focused on (1) whether there was loss of transparency (that is, whether there was turbidity), (2) the surface properties, (3) the presence/absence of white spots, and (4) the presence of characteristic findings. The significant findings were selected, but when it was clear that there were mixed findings, the specimen was judged as mixed. It was approved by the Ethics Committee of Tokushima University School of Medicine. For this type of study formal consent is not required.

Received for publication October 4, 2021; accepted October 25, 2021.

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Figure 1. Macroscopic findings for a VAB specimen of a normal mammary gland. The normal mammary gland is rather transparent, and the surface appears smooth under tangential light. (normal mammary gland).

SUBJECTS

From January 2018 through December 2018, VAB was performed for 102 patients at Tokushima Breast Care Clinic. VAB was performed under ultrasound-guided local anesthesia by a single surgeon using a breast biopsy device, Celero-12 (Hologic Ltd.; Manchester, UK). Based on the image findings and histopathological results, all but one case was judged to have been properly biopsied. The reasons for performing VAB were as follows: in 25 cases, fine needle aspiration biopsy (FNA) was suspicious for malignancy; in 44 cases the FNA was indeterminate; in 17 cases, imaging was suspicious for malignancy, but FNA indicated benign; in 5 cases, imaging was suspicious for malignancy, but FNA was not performed; in 5 cases, MRI examination performed before breast cancer surgery indicated a new lesion, and 2nd-look US detected lesions; and in 5 breast cancer cases, VAB was required for subtype determination. The age range was 32 to 81 years (mean, 51.7 years), and the lesion size was 3.4 to 27 mm (mean, 11.4 mm).

Macroscopic findings for VAB specimens

The VAB specimens were immediately photographed with a smartphone. The imaging method was the same as that described above for surgical specimens. The macroscopic findings were later evaluated by two doctors (one surgeon and one radiologist), and a consensus evaluation was reached. The evaluated features were: (1) presence/absence of turbidity (turbidity, no turbidity, mixed, difficult to evaluate); (2) surface properties (smooth (smooth and/or shiny), rough, mixed, difficult to evaluate); (3) presence/absence of white spots; and (4) characteristic findings (papillary structure, circular granular structure, contraction, edema, red cord-like structure, presence of mucus).

Examination for relationship between VAB specimen macroscopic findings and pathological results (benign/malignant)

The pathological results for VAB specimens were divided into two categories, malignant and benign, and examined for a relationship with the macroscopic findings. In addition, benign disease specimens were examined for a relationship between turbidity/no turbidity and the histopathological result. Furthermore, the characteristic findings were examined for a relationship with the histopathological result. Statistical testing used the χ^2 test, and $p < 0.05$ was defined as significant.

RESULTS

Relationship between turbidity and pathological results (benign/malignant)

Evaluation was possible for 99 of the 101 (98%) cases. Malignancy was detected in 33/37 (89%) specimens with turbidity (Fig. 2a), but in only 2/47 (4%) specimens without turbidity (Fig. 2b); the difference was statistically significant ($P < 0.001$) (Table 1). Moreover, malignancy was found in 5/15 (33%) mixed specimens, and the difference with the turbid specimens was clearly significant ($p < 0.001$). For the benign specimens, 73% of intraductal papillomas (IDP) and 67% of ductal hyperplasia (DH) showed no turbidity, and none of the fibroadenomas (FA) showed turbidity. The differences were not significant (Table 2).

Relationship between surface properties (rough/smooth) and pathological results (benign/malignant)

Evaluation was possible for 99 (98%) specimens. There were 70 smooth specimens, of which 14 (19%) were malignant, whereas 24/29 (83%) rough specimens (Fig. 2c) were malignant; the difference was statistically significant ($p < 0.001$) (Table 1). No specimens were judged to be mixed.

Table 1. Relationship of the macroscopic findings (turbidity, surface properties, white spot) with pathological results (benign/malignant)

		benign	malignant	
turbidity	presence	4	33	p<0.001
	absence	45	2	
	mixed	10	5	p<0.001
	difficult to evaluate	2	0	
surface properties	rough	5	24	p<0.001
	*smooth	56	14	
	mixed	0	0	
	difficult to evaluate	0	2	
white spot	presence	2	11	p<0.001
	absence	59	29	
	difficult to evaluate	0	0	

*smooth and/or shiny

Table 2. Relationship between turbidity and histopathological results (benign case)

turbidity	IDP ^a	DH ^b	FA ^c
presence	1	3	0
absence	16	18	11
mixed	5	5	0
difficult to evaluate	0	1	1
	22	27	12

a: intraductal papilloma, b: ductal hyperplasia, c: fibroadenoma

Relationship between the macroscopic findings and pathological results (benign/malignant) with consideration of turbidity and the surface properties (rough/smooth)

Evaluation was performed for 100 specimens after excluding one specimen that consisted of a mucus mass, 21/22 (96%) turbid specimens with a rough surface were malignant, whereas malignancy was found in only 4% of specimens that were not turbid and had a smooth surface (Table 3). Sixty-one percent (22/36) of the turbid specimens had a rough surface, while 96% (45/47) of the specimens without turbidity were smooth, indicating a clear relationship between the presence/absence of turbidity and the surface properties (data not shown).

Relationship between white spots and pathological results (benign/malignant)

All 101 specimens were evaluated for white spots. White spots were observed in 13 specimens (Fig. 3a), of which 11 (85%) were malignant. The remaining 88 specimens had no white spots,

and 29 (33%) were malignant. The difference was statistically significant ($p < 0.001$) (Table 1).

Relationship between the combination of turbidity, surface properties rough, and white spot presence and malignant percentage

For the three factors that showed significant differences (turbidity, roughness of surface properties, and presence of white spots), the percentage of malignancy by the number of points was determined. Any two factors were associated with a high malignancy rate and three factors were 100% malignant (Table 4).

Relationships of the characteristic findings with pathological results (benign/malignant) and the histopathological results

Table 5 shows the characteristic findings and the histopathological results. As the main characteristic findings, 37 specimens had a papillary structure (Fig. 3b), 33 specimens had a circular granular structure (Fig. 3c), 12 specimens showed contraction (Fig. 3d) and 9 specimens showed edema (Fig. 3e). With regard to the relationship between the characteristic findings



Figure 2.
 a : Macroscopic findings for a VAB specimen ; turbidity. Decreased light transmission indicates the presence of turbidity. Bleeding is also present. A papillary structure is seen (non-invasive ductal carcinoma).
 b : Macroscopic findings for a VAB specimen ; no turbidity. A papillary structure is seen (intraductal papilloma).
 c : Macroscopic findings for a VAB specimen ; rough surface. Fine-sand-like, light-reflecting spots are observed under tangential light, and surface irregularities are present overall (invasive ductal carcinoma).

Table 3. Relationship between the macroscopic findings and pathological results (benign/malignant) with consideration of turbidity and surface properties (rough/smooth)

turbidity	presence		absence		mixed	
	rough	smooth	rough	smooth	rough	smooth
cases	22	14	2	45	5	10
malignant (%)	21 (96)	11 (77)	0 (0)	2 (4)	3 (60)	2 (20)

Table 4. Relationship between the combination of turbidity, surface properties rough, and white spot presence and malignant percentage

	1 factor	2 factors	3 factors
turbidity presence	33/37 (89%)	+ rough 21/22 (95%) + white spot 10/10 (100%)	
surface properties rough	24/29 (93%)	+ white spot 7/7 (100%)	7/7 (100%)
white spot presence	11/13 (85%)		
total	68/79 (86%)	38/39 (97%)	7/7 (100%)

and pathological results (benign/malignant), malignancy was found in 42% (14/33) and 50% (6/12), respectively, of the circular granular structure specimens and the contracted specimens. With regard to the histopathological result, more than half of the IDP specimens had a papillary structure, and 32% were malignant. FA specimens were characterized by edema and red cord-like structures (Fig. 3e). Mucus was present in only one specimen, and that case was mucinous cancer.

DISCUSSION

Today, needle biopsy is an essential technique for preoperative diagnosis of breast cancer. One of the major causes of false negatives in needle biopsy diagnosis is that the lesion was not properly biopsied (6-8). If the macroscopic findings of the needle biopsy specimen match the expected histopathological results, it can be judged that the needle biopsy was properly performed. Our literature search found that Rosen, *et al.* performed detailed

Table 5. Relationships of the characteristic findings with the pathological results (benign/malignant) and the histopathological results

	total	FA ^a	IDP ^b	DH ^c	DCIS ^d	IDC ^e
papillary structure	37	1	20	3	5	7
circular granular structure	33	3	2	11	4	10
contraction	12	0	1	4	0	6
edema	9	9	0	0	0	0
red core-like structure	5	5	0	0	0	0
mucus	1	0	0	0	0	1

a : fibroadenoma, b : intraductal papilloma, c : ductal hyperplasia, d : ductal carcinoma in situ, e : invasive ductal carcinoma

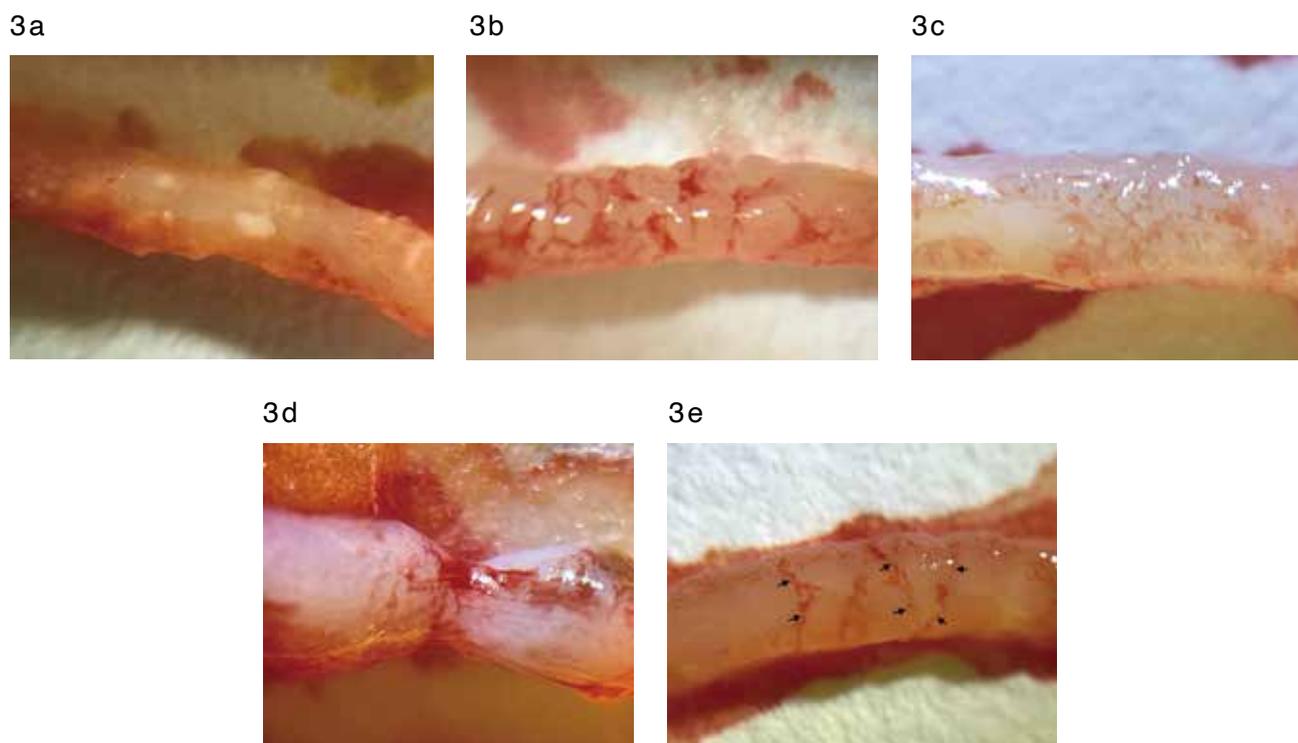


Figure 3.

a : Macroscopic findings for a VAB specimen ; white spots. White to pale yellow spots of <1 mm are being extruded from the mammary gland tissue (non-invasive ductal carcinoma).

b : Macroscopic findings for a VAB specimen ; papillary structure. A structure of relatively large circles of 1-2 mm, with an overall papillary shape, is seen (ductal hyperplasia).

c : Macroscopic findings for a VAB specimen ; circular granular structure. Circular granular structures of less than 0.5-1 mm are observed. They are smaller than papillary structures and overall do not have a papillary shape (non-invasive ductal carcinoma).

d : Macroscopic findings for a VAB specimen ; image of contraction. An image of contraction accompanied by stenosis and distortion is seen. Occasionally, radial white and red fibrous structures are observed (invasive ductal carcinoma).

e : Macroscopic findings for a VAB specimen ; red cord-like structures and edema. Mucus-like or gelatinous nodules are separated by red cord-like structures (arrow). Transparent edema is also present (fibroadenoma).

examination of the gross findings for excised fresh specimens (12), but we found no reports of studies of needle biopsy specimens (9). Our present report is thus the first to examine needle biopsy specimens in detail.

This was a retrospective study. Specimens were photographed with an iPhone. This is because the number of pixels of the iPhone camera, 12 million, was judged to be sufficient for documenting the specimens properties, while the camera can be easily used in ordinary facilities. A commercial, readily-available illumination device was used for tangential lighting. Rosen PP had used tangential light to observe excised specimens, and we judged that tangential light was suitable for accurately observing the surface properties of our needle biopsy specimens. In addition, we examined VAB specimens because larger specimens can be evaluated compared to core needle biopsy (CNB) specimens.

As macroscopic findings, we examined only the 1) presence/absence of turbidity, 2) surface properties, 3) presence/absence of calcification and 4) characteristic findings. Turbidity was examined because it has been thought to be associated with an increase in cell components. The surface properties were examined because they have been thought to reflect the influence of tumors on the stroma. White spots were examined because they are thought to represent microcalcifications and necrosis. Our terminology for the characteristic findings was based on the terminology used for gastrointestinal endoscopic findings (13). The findings were retrospectively reviewed and classified. We did not evaluate the color of our VAB specimens because Rosen PP reported that, for their excised specimens, even invasive ductal carcinoma (IDC) exhibited a range of colors (white, gray and yellow) (12). Regarding the pathological results, because the number of evaluated cases was small, all the cases of breast cancer were considered malignant, and we did not examine for a relationship with the histopathological result. On the other hand, for cases of benign disease, we did examine the presence/absence of turbidity and the characteristic findings for relationships with the histopathological result.

Our results indicate that malignancy was significantly more frequent in specimens with turbidity and a rough surface. Furthermore, the malignancy rate was 96% for specimens with both turbidity and a rough surface. In addition, only 13% of our specimens had white spots due to microcalcifications and necrosis, but their rate of malignancy was significantly high. Collectively, specimens showing turbidity, specimens with a rough surface, and specimens with white spots were significantly more likely to be malignant. Also, the presence/absence of microcalcifications and necrosis was able to be confirmed by visual observation.

Next, specimens with a circular granular structure as a characteristic finding had a malignancy rate of 42%, while the rate was 50% for specimens with contraction. Although papillary structures are common in IDP, 32% were malignant. In addition, edema and red cord-like structures were found to be characteristic of FA. In Rosen PP study using fresh samples, radial sclerosing lesions, which are benign disease, had a retracted center with white streaks, while cases with adenosis had abundant calcifications and appeared to be gritty. On the other hand, for IDC they wrote that “the appearance of the cut surface very considerably depended on the composition of the tumor,” etc. (12). Excised specimens and VAB specimens differ not only in size: it is predicted that excised specimens often include normal mammary gland tissue, and the macroscopic findings are also expected to differ between these two types of specimen. In the future it will be necessary to investigate VAB specimens for a relationship between their macroscopic findings and histopathological results.

When needle-biopsy of a lesion that is targeted for resection

yields macroscopic findings that match the predicted histopathological findings, it can be thought that the biopsy had been properly performed. If we confirm the predicted macroscopic findings, the sampling is completed with two. In the case that there is no match, additional needle biopsy can be performed. Therefore, even if the number of samples is two, we think that false-negatives due to poor specimens can be prevented by examining needle-biopsy macroscopic findings.

This study has a number of limitations. (1) The results of the dedicated imager and smartphone should be compared and examined, but we did not do this. (2) Turbidity and surface properties are subjective evaluations, and a multi-institutional reproducibility study is needed. (3) It is necessary to investigate whether CNB specimens can also be used. (4) This was a retrospective study, and a prospective study needs to be carried out.

CONCLUSIONS

When needle-biopsy of a lesion that is targeted for resection yields macroscopic findings that match the predicted histopathological findings, it can be thought that the biopsy had been properly performed. This means that false-negatives due to poor specimens can be prevented. In the future, it will be necessary to conduct reproducibility and prospective studies at multiple institutions.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

Not applicable

ETHICS DECLARATIONS

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Tokushima University School of Medicine, case number No.3535. For this type of study formal consent is not required.

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