Modified Core Wash Cytology (CWC), an Asset in the Diagnostic Work-up of Breast Lesions

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Condensed Abstract
The modified core wash cytology procedure is a valuable tool for obtaining a rapid diagnosis from core needle biopsies of breast lesions.
Abstract

Aim: A quick and reliable preliminary diagnosis is essential in the management of a same-day breast clinic. In a preclinical study we developed an alternative method of core wash cytology (CWC). This study is an evaluation of this new CWC method introduced into the clinical setting.

Methods: From April 2008 to April 2009, biopsies were taken from lesions in the breast. CWC was obtained from core needle biopsy (CNB) with a modified technique and classified into the categories: malignant, suspicious for malignancy, atypical, benign and inadequate. CWC and CNB diagnoses were correlated with the histopathology of subsequently obtained resection specimens. The sensitivity and specificity were calculated.

Results: CWC was obtained from 226 breast lesions. In 167 of these cases subsequent resection of the lesion was performed revealing 149 carcinomas and 18 benign lesions. Of the 149 malignant cases, 136 were considered as either malignant or suspicious for malignancy by CWC, 7 as atypical, 4 as benign and 2 as inadequate. None of the 18 benign lesions were classified as suspicious or malignant on CWC. Eight out of 149 resected carcinomas were not recognized as malignant by histological analysis of the CNB, while 7 of these cases the CWC was considered malignant. The sensitivity and specificity were 97% and 100%, respectively.

Conclusions: In the vast majority of patients the modified CWC technique can provide a quick and reliable diagnosis of malignant breast lesions. Furthermore, combining CWC with CNB histology can improve adequate, preoperative recognition of the malignant character of breast lesions.

Keywords: core wash cytology, breast clinic
Introduction

The core needle biopsy (CNB) is increasingly being used as a first-line diagnostic modality in the diagnostic work-up of palpable and non-palpable breast lesions. Indeed, histological examination of such biopsies generally provides very important and detailed information about tumour characteristics, including subtype of carcinoma, hormonal status and other molecular features.

However, a same-day breast clinic requires a quick and reliable diagnosis for same-day patient counselling and optimal planning of further surgical or neo-adjuvant management. In most laboratories, a CNB diagnosis takes 12-24 hours of processing. Core wash cytology (CWC) or touch imprint cytology (TIC) could be a solution for this problem because by cytological analysis a diagnosis can often be rendered within one hour. Unfortunately, the results described in the literature with such cytological diagnostic approaches are variable. In studies on CWC the sensitivity and specificity varied from 85% to 89% and 72% to 98%, respectively, while the inadequate rate varied from 7% to 42% [1,2]. Studies on TIC showed variable results as well [3-10].

We developed a modified CWC technique in which the collecting fluid is optimized and the processing is standardized. This technique was first tested in a laboratory setting and yielded very promising results, especially in malignant lesions [11]. We then introduced the CWC approach into the clinical setting.

In this manuscript we present the results obtained in the first year after the introduction of the modified core wash technique.

Materials and methods

Patient and tissues samples
From April 2008 to April 2009, CNBs were taken from palpable and non-palpable lesions in the female breast in our teaching hospital. According to the Breast Imaging Reporting and Data System (BI-RADS), developed by the American College of Radiology, biopsies were taken by the radiologist. Two to 3 CNBs per lesion were obtained by radiologists, using an 18 gauge needle (Bard Peripheral Vascular, Inc., Tempe, Arizona USA) and biopsy gun under ultrasound guidance.

**CWC technique**

The biopsies were collected in 6 ml RPMI® medium (Roswell Park Memorial Institute, Invitrogen, Breda, The Netherlands). To each 500ml RPMI 0.5 gr. Natrium-azide and 5 gr. Bovine Serum Albumin (Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands) was added. The biopsies were kept in the medium for 5-10 minutes, and additionally the needle tip was rinsed in the medium. Subsequently, the needle biopsies were removed from the medium by a cytological technician, fixed in formalin, paraffin embedded and haematoxylin and eosin stained (Fig. 1).

The CWC was performed by fixing the remaining cells in the medium for 10 minutes in 2ml Fixcyt® (50 % ethanol/ polyethylene glycol 2% solution). According to the operator’s manuals, this mixture was centrifuged for 10 min at 688 rpm in a Hettich Rotina 48S® or Hettich Rotanta 46S® centrifuge (Andreas Hettich GmbH & Co.KG, Tüttlingen, Germany).

Next, the supernatant was discarded and dependent on the estimated density of the sediment, 1 or 2 drops of the Fixcyt® solution were added. The resultant mixture was collected in a container and then put on a poly-L-lysine slide (Menzel GmbH, Braunschweig, Germany). By centrifuging for 5 min at 688 rpm, the sediment was pressed onto the slide, creating a
monolayer arrangement of all cells within a 12 mm diameter area [12,13]. The liquid based preparation slides were Papanicolaou-stained (Fig. 1). The preparation and the screening of the CWC slides was done by a cytotechnologist and supervised by a cytopathologist (total group in our centre consisting of 6 cytotechnologists and 8 pathologists with ample experience in cytopathology).

The clinician was informed about the CWC diagnosis within one hour after puncture and about the histological diagnosis on CNB material early in the following morning.

**Classification**

The cytological criteria for CWC are exactly the same as those used for fine needle aspiration (FNA). The presence of more than 6 epithelial cell clusters was required for adequate classification.

According to international guidelines for FNA cytology, the CWC findings were categorized as benign, atypical, suspicious for malignancy, malignant or inadequate. Based on our preclinical study, on CNB, we considered carcinoma, suspicious for carcinoma and ductal carcinoma in situ (DCIS) as “positive for carcinoma” [11]. Not representative and no material were put in the inadequate category.

The results of the CWC were correlated with the histopathology of the CNB and, when available, subsequent resection specimens. The CWC test was considered as “conclusive” when the diagnosis on CWC was malignant, suspicious for malignancy or benign. Sensitivity was defined as the proportion of malignant, suspicious for malignancy CWC cases divided by malignant (DCIS, suspicious for carcinoma and carcinoma) cases on the final resection specimen. Specificity was defined as the number of benign CWC cases divided by non-malignant cases on histology of the final resection specimen.
If the CWC diagnosis was malignant or suspicious for malignancy, the patients were planned for further surgical or neo-adjuvant management.

Results

Patients and Pathological findings

In a one year period (April 2008- April 2009), CWC was obtained from the CNB of 226 breast lesions. The age of the patients ranged from 21-92 years (median 57.5 years). In 167 of these cases subsequent resection of the lesion was performed in the follow-up.

Malignant cases

The resection specimens revealed 149 carcinomas (ductal \(n = 111\), lobular \(n = 24\), DCIS \(n = 7\) (one grade 2 and 6 grade 3), mucinous \(n = 4\), tubular \(n = 1\), medullary \(n = 1\), papillary \(n = 1\)). Of the 149 malignant cases, by CWC 136 cases were classified as either malignant \((n = 110)\) or suspicious for malignancy \((n = 26)\), 7 as atypical, 4 as benign and 2 as unsatisfactory (Table 1). Of the 111 cases of ductal carcinoma in the final specimen histology the CWC was “positive” in 105 cases (malignant \(n = 92\); suspicious for malignancy \(n = 13\) ), atypical in 4 cases, benign in one case, and inadequate in one case. In 4 of these 111 cases the CWC diagnosis was malignant and the CNB diagnosis was inadequate \((n = 3)\) or benign \((n = 1)\). In the group of 24 lobular carcinomas CWC was “positive” in 18 cases (malignant \(n = 11\); suspicious for malignancy \(n = 7\) ), atypical in 3 cases, benign in 2 cases, and one case was non-diagnostic due to inadequate cytological material. In this lobular carcinoma group the histological CNB diagnosis was malignant in all cases.
In the group of 7 cases with DCIS, the final histology revealed one case with ductal carcinoma in situ grade 2, which was suspicious for malignancy on CWC and inadequate on CNB. Of the 6 DCIS grade 3 cases, one case revealed a benign diagnosis on CWC with insufficient diagnostic tissue on CNB. Two of three malignant cases on CNB were also malignant on CWC, one was suspicious for malignancy on CWC. However, two cases revealed the diagnosis suspicious for malignancy on CWC but the CNB was inadequate histological material and did not allow for a diagnosis on CNB.

The 7 mucinous, medullary, tubular and papillary carcinomas were all “positive” on CWC (malignant $n = 5$, suspicious for malignancy $n = 2$) and CNB.

For confirmation of the CWC diagnosis in one of the 4 ductal carcinoma cases another (second) CNB was taken and in all DCIS cases.

**Benign cases**

Eighteen resected breast lesions were benign including 4 cases of adenosis, 9 benign fibrous tumours, one intracystic papilloma, one case of mastitis, 2 of fat necrosis, and one of mammary duct ectasia (Table 2). On CWC, none of these benign lesions were classified as suspicious for malignancy or malignant, 8 lesions were classified as benign, 7 as atypical, and 3 as inadequate.

The group of 9 fibrous tumours consisted of 6 fibroadenomas which were diagnosed as benign in 3 cases, and as atypical in 3 other cases on CWC. Two of the fibrous tumours were phyloides tumours on final histological analysis, one case was diagnosed benign in both CWC and CNB histology and in the other case a diagnosis of atypia was given in both techniques. The ninth fibrous tumour was a desmoid fibromatosis which was diagnosed benign on CNB and was inadequate on CWC.
The intracystic papilloma was diagnosed benign on CNB, however showed atypia on CWC. The cases with fat necrosis, mastitis and duct ectasia in the final specimen histology were benign in both the CWC and CNB histology.

The CWC test was considered as “conclusive” (malignant, suspicious for malignancy or benign) in 88% of the cases. The sensitivity and specificity of the CWC diagnosis compared to the definite resection diagnosis was 97% (95% confidence interval: 93%-99%) and 100% (95% confidence interval: 59%-100%), respectively. The percentage of CWC examinations resulting in the diagnosis inadequate was 4.0%.

Cases without immediate biopsy or resection

In 59 cases no subsequent resection was performed. (Table 3) In 26 cases the CNB was malignant and in 33 cases benign. Of these 26 malignant cases, by CWC 24 cases were classified as either malignant \( n = 21 \) or suspicious for malignancy \( n = 3 \), and in 2 cases atypia was suggested on CWC.

Fifteen of these patients with malignant tumours without subsequent resection of the breast lesion underwent neo-adjuvant chemotherapy (mean age 45.6 years), 10 cases received hormonal therapy (mean age 83.6 years in this subgroup), and one patient had a diffuse large B-cell Non Hodgkin lymphoma (which was diagnosed as atypia on CWC) and received chemotherapy.

In none of the 33 cases with a benign CNB diagnosis a CWC diagnosis malignant or suspicious for malignancy was rendered. In these 33 cases, follow up for pathological diagnosis by the Dutch Network and National Database for Pathology (PALGA), at least 6 months after the CNB still did not reveal malignancy, supporting the benign nature of the breast lesion in these 33 patients.
Discussion

Management breast clinic

For optimal organization of a same-day breast clinic a quick and reliable pathological diagnosis is warranted. A histological diagnosis in 1 hour, at all times of the day, would be the best but is until today not available. Touch imprint cytology (TIC) or core wash cytology (CWC) could be a solution in a same-day breast clinic, but variable results were described for both techniques in the literature. A recent study on TIC revealed a sensitivity and specificity of 97.7% and 94.2%, respectively [10]. Interestingly, in that study the inadequate cases (5%) were also excluded from the calculations, but in contrast to our study the cytological diagnosis atypia was included in the benign group. Our study shows that in case of the cytological diagnosis atypia further examination is needed to rule out malignancy.

Modified CWC technique

In a preclinical study we obtained very promising results with a modified CWC technique generating highly informative cytological material from CNBs [11]. Therefore, we introduced this modified technique in the clinical setting. Both in the preclinical and clinical study, the specificity of a “conclusive” CWC diagnosis was 100%. The sensitivity for this diagnosis was in this clinical study 97% and in the preclinical study 95%, respectively. The percentage of cases with a CWC diagnosis inadequate was comparable in the clinical and preclinical study (4.0% vs. 6.6%). As we stated in the preclinical study, it is important to note that the quality of the results on CWC are for a major part determined by factors such as the experience of the pathologists, the nature and the quality of the collecting medium, the standardised handling of the material by the technicians, and the cytopsin monolayer preparation [3,11,13].
Literature CWC

The recent CWC study of Uematsu et al. on 458 consecutive patients with breast lesions showed a sensitivity and specificity of 89 and 72%, respectively, while 42.2% of the samples were considered as inadequate [2]. Also in that study inadequate samples were more often obtained from benign breast lesions which is probably due to the nature of these lesions. One older study describes similar diagnostic results: 7% inadequate samples on CWC and a sensitivity and specificity of 85 and 98%, respectively [1]. Of note, Lankford et al used a larger (16 gauge) core needle, while the 18 gauge needle we used may be more comfortable for the patient.

Findings from this study

Our present clinical study confirms the observations of our preclinical study [11]. In the malignant group, seven cases were classified as atypical on CWC (4 of 111 ductal and 3 of 24 lobular carcinoma cases). In these cases the biopsies were generally very short and/or fragmented, and a smaller volume of biopsy material may very well result in a lower number of cells available for cytological diagnosis. Also, the percentage of the diagnosis atypia was significantly higher in the group of lobular carcinomas than in ductal carcinoma cases. This might be explained by the fact that lobular carcinoma cells often show only discrete nuclear atypia and can also mimic lymphocytes in cytological specimens. Cells of lobular carcinomas may therefore be more difficult to recognize as malignant cells, especially when few cells are available for cytological examination.
The 4 cases that were (also retrospectively) classified as benign on CWC and as malignant on CNB all had relatively few cells available for cytological examination but did meet the requirements for adequate material.

*Extra diagnostic modality*

Interestingly, in 8 cases with a final diagnosis of malignancy in the resection specimen, the histological examination of the CNB did not reveal malignancy. Of these, 4 ductal carcinoma cases yielded inadequate material for diagnosis on CNB \((n = 3)\) or a benign diagnosis \((n = 1)\) on CNB. All these 4 cases were malignant on CWC. Additionally, 4 out of the 7 DCIS cases revealed inadequate material after tissue processing for a CNB histological diagnosis (only a minimal amount of tissue fragments could be recovered from the RPMI fluid), while in 3 of these 4 cases the CWC diagnosis was malignant. A potential drawback of introducing CWC is that already small and vulnerable tissue specimens become even less suitable for subsequent histological analysis. However, disintegration of vulnerable tissue can already occur during transportation of the material to the pathology laboratory and during preparation for histological analysis anyway. Interestingly, in one of our cases material of two CNB’s was submitted for pathological analysis, but one of these specimens was fragmented and lost for further histological analysis, while the other histologically only contained benign breast tissue. In this case the CNB diagnosis was ‘benign’, but the CWC diagnosis was ‘malignant’, probably because tumour cells were shed from the fragmented biopsy specimen into the liquid that was used for CWC analysis. As stated before, it is uncertain if such fragments would have yielded a proper diagnosis if they were used for only histological analysis. In a substantial part of these cases, 7 out of 8 cases, a CWC diagnosis was rendered which allowed further management of the patients.
Five out of these 7 cases underwent a second CNB for confirmation of the CWC diagnosis.

**Benign lesions**

This present study included 51 benign lesions (18 proven by histology on resection specimens and 33 cases with clinical but without histological follow-up). None of the benign cases in our study, however, had the diagnosis suspicious for malignancy or malignant on CWC, while in 9 cases the diagnosis was atypia on CWC.

**Patients planning**

In our breast clinic we have the agreement to plan patients with a diagnosis malignant and suspicious for malignancy on CWC. Based on the results obtained in this study and the local agreement we created a flow chart illustrating the role of the CWC diagnosis in the management of patients in a same-day breast clinic (Fig. 2). The high sensitivity and specificity of a “conclusive” (malignant/suspicious for malignancy and benign) diagnosis on CWC allows for rapid further planning of the vast majority of patients.

**Conclusion**

We conclude that, combining CNB with CWC analysis for the initial diagnostic workup of breast lesions has some clear advantages: 1. the modified CWC technique can provide a quick and reliable diagnosis of (especially malignant) breast lesions, which is valuable for same-day patient counselling and management planning; 2. combining CWC with CNB findings can lead to an increase in the number of adequate preoperative diagnoses of malignancy in breast lesions.
References


Table 1
Core wash cytology (CWC) diagnoses (in bold) and core needle biopsy CNB histological diagnosis (in *italics*) in relation to the final histological diagnoses of malignant lesions in resection specimens

<table>
<thead>
<tr>
<th>Final malignant diagnoses</th>
<th>Malignant/ suspicious for malignancy</th>
<th>Atypia</th>
<th>Benign</th>
<th>Inadequate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductal carc. <em>n</em> = 111</td>
<td>105/107</td>
<td>4/0</td>
<td>1/1</td>
<td>1/3</td>
</tr>
<tr>
<td>Lobular carc. <em>n</em> = 24</td>
<td>18/24</td>
<td>3/0</td>
<td>2/0</td>
<td>1/0</td>
</tr>
<tr>
<td>DCIS <em>n</em> = 7</td>
<td>6/3</td>
<td>0/0</td>
<td>1/0</td>
<td>0/4</td>
</tr>
<tr>
<td>Other malignant lesions <em>n</em> = 7</td>
<td>7/7</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Total <em>n</em> = 149</td>
<td>136/141</td>
<td>7/0</td>
<td>4/1</td>
<td>2/7</td>
</tr>
</tbody>
</table>
Table 2
Results core wash cytology (CWC) diagnosis (in bold) and core needle biopsy (CNB) (in *italics*) relation to the final benign diagnoses in resection specimens

<table>
<thead>
<tr>
<th>Final benign diagnoses</th>
<th>Malignant/ suspicious for malignancy</th>
<th>Atypia</th>
<th>Benign</th>
<th>Inadequate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosis ( n = 4 )</td>
<td>0/0</td>
<td>2/0</td>
<td>0/4</td>
<td>2/0</td>
</tr>
<tr>
<td>Fibrous tumours ( n = 9 )</td>
<td>0/0</td>
<td>4/1</td>
<td>4/7</td>
<td>1/1</td>
</tr>
<tr>
<td>Intracystic papilloma ( n = 1 )</td>
<td>0/0</td>
<td>1/0</td>
<td>0/1</td>
<td>0/0</td>
</tr>
<tr>
<td>Others ( n = 4 )</td>
<td>0/0</td>
<td>0/0</td>
<td>4/4</td>
<td>0/0</td>
</tr>
<tr>
<td>Total ( n = 18 )</td>
<td>0/0</td>
<td>7/1</td>
<td>8/16</td>
<td>3/1</td>
</tr>
</tbody>
</table>
### Table 3
Results of core wash cytology (CWC) and core needle biopsy (CNB) of the 59 cases with only ‘archival’ follow-up confirmation of the diagnosis

<table>
<thead>
<tr>
<th>Final diagnosis</th>
<th>CWC</th>
<th>CNB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malignant/ Atypia Suspicious Benign Inadequate Malignant Atypia Benign Inadequate</td>
<td>Malignant Atypia Benign Inadequate</td>
</tr>
<tr>
<td>Malignant (adjuvant therapie) n = 15</td>
<td>15/0</td>
<td>0</td>
</tr>
<tr>
<td>Malignant (elderly) n = 10</td>
<td>6/3</td>
<td>1</td>
</tr>
<tr>
<td>Lymphoma n = 1</td>
<td>0/0</td>
<td>1</td>
</tr>
<tr>
<td>Benign n = 33</td>
<td>0/0</td>
<td>7</td>
</tr>
<tr>
<td>Total n = 59</td>
<td>21/3</td>
<td>9</td>
</tr>
</tbody>
</table>
Figure 2

Day 1

CWC Diagnosis

- 'Conclusive' (sensitivity 97%, specificity 100%)
- 'Inconclusive'

Malignant/ Suspicious for malignancy

Planning same-day

Benign

No planning necessary, finished

Atypia/ Inadequate

Day 2

(Confirmation by CNB Diagnosis)

(Confirmation by CNB Diagnosis)

Day x

CNB Diagnosis

Separate consult for patient planning