

**Research Article**

**Analysis of Cell Block and Cytology Specimen Preservation from Lung Aspiration Biopsy**

**Analisis Preservasi Spesimen Hapusan Sitologi dan Cell Block pada Bahan Biopsi Aspirasi Paru**

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**ABSTRACT**

*Cytology smear technique is often used in Indonesia because the process is safe, simple, easy, fast, and cost effective. At present, several studies have found that smears with cell block techniques are of better quality than smears with cytology smear techniques. The aim of this study was to analyze whether the cytology smear technique can produce adequate specimens compared to cell block towards results of lung Fine Needle Aspiration Biopsy (FNAB). Lung FNAB specimens were divided into two parts: one part was processed with cytology and the other part with cell block technique. The specimens were observed under a microscope to count the number of inflammatory cells and the number of artifacts. The numbers of inflammatory cells and artifacts were scored 0-3. The inflammatory cells consisted of neutrophils, lymphocytes and plasma cells, also macrophages. The result showed no significant difference between the number of inflammatory cells in cytology and cell block ( $p$  neutrophils=0.543;  $p$  lymphocytes and plasma cells=0.192;  $p$  macrophages=0.487) in 38 samples. The artifact score comparison test result showed a significant difference between the number of artifacts in cytology and cell block ( $p=0.027$ ) with more artifacts in cytology. The most common artifact in cytology was air bubble artifacts, while cell block was dominated by torn pieces artifacts. There was no significant difference between the number of inflammatory cells found in cytology and cell block techniques. Cell block technique has less artifacts than cytology, but artifacts found in cytology can be corrected so that the cytology smear technique is still an option.*

**Keywords:** Artifacts, cytology, cell block, inflammatory cells

**ABSTRAK**

Teknik sitologi merupakan teknik pemeriksaan spesimen yang banyak digunakan di Indonesia sebab prosesnya mudah, cepat, dan murah. Beberapa penelitian saat ini menyatakan bahwa spesimen yang diproses dengan teknik *cell block* memiliki kualitas yang lebih baik dibandingkan sitologi. Penelitian ini bertujuan untuk menganalisis preservasi spesimen yang diproses dengan teknik sitologi dan *cell block* terhadap hasil biopsi aspirasi paru. Spesimen hasil FNAB paru dibagi menjadi dua bagian: satu bagian diproses dengan sitologi dan bagian lainnya dengan teknik *cell block*. Preparat diamati di bawah mikroskop untuk menghitung jumlah sel radang dan jumlah artefak. Selanjutnya dihitung jumlah sel radang yang terdiri dari neutrofil, limfosit dan sel plasma, serta makrofag dan jumlah artefak dalam skor 0-3. Hasil uji komparasi pada 38 sampel tidak ditemukan perbedaan yang bermakna antara jumlah sel radang pada sitologi dan *cell block* ( $p$  neutrofil=0,543;  $p$  limfosit dan sel plasma=0,192;  $p$  makrofag=0,487). Sedangkan pada hasil uji komparasi skor artefak didapatkan bahwa terdapat perbedaan yang bermakna antara jumlah artefak pada sitologi dan *cell block* ( $p=0.027$ ) dengan jumlah artefak pada sitologi lebih banyak dibandingkan *cell block*. Jumlah artefak dominan pada sitologi adalah artefak gelembung udara, sementara pada *cell block* didominasi oleh artefak potongan sobek. Tidak ada perbedaan yang bermakna antara jumlah sel radang yang ditemukan pada teknik sitologi maupun *cell block*. Pada jumlah artefak, kualitas *cell block* lebih baik dibandingkan sitologi. Artefak pada sitologi dapat diperbaiki sehingga teknik sitologi masih menjadi pilihan terutama pada pusat kesehatan yang tidak memiliki peralatan yang memadai.

**Kata Kunci:** Artefak, *cell block*, sel radang, sitologi

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DOI: <http://dx.doi.org/10.21776/ub.jkb.2020.031.01.5>

## INTRODUCTION

Cellular preservation is an important factor for provision of adequacy specimen evaluation (1). It was done by observing the morphology of inflammatory cells. Inflammatory cells are always found in every cytopathologic specimen and one of the criteria for cytopathologic specimen adequacy taken using Fine Needle Aspiration Biopsy (FNAB) in the thorax is by finding the inflammatory cell of alveolar macrophage type (2) so that the inflammatory cells are used as indicator of cell preservation.

Pre-operative analysis techniques for tumor cells currently used are cytology and cell block. Cytology smear technique is often used in Indonesia because the process is safe, simple, easy, fast, and cost effective (3). At present, several studies have found that smears with cell block techniques are of better quality than smears with cytology smear techniques because they are fixed immediately so as to avoid air drying artifacts (4). In Indonesia cell block smear technique is rarely used, although this technique is often considered to be superior to cytology because cell block technique has some disadvantages. Cell block processing requires a longer time than cytology (5). In addition, the processing requires special skills and adequate equipment so that it requires greater costs for equipment, human resources, and adds an extra cost to patient management (6), so that health centers that do not have adequate personnel and equipment rely solely on cytology as a specimen processing technique.

Cytology smear technique is currently the main choice in Dr. Soetomo Hospital, Surabaya, Indonesia because cytological smear result is of high-quality and it is a superior technique that is still used today. This is not in accordance with previous studies which found that smear with cell block technique has better quality than cytology in the aspects of the number of inflammatory cells and cellularity (7,8). However, those previous studies had the disadvantage that their specimens were only counted and compared in one visual field, while cytology and cell block techniques have different principles so they cannot be compared in only one visual field. In this study we converted the number of inflammatory cells in one visual field into scoring system so that it can represent the entire visual field.

We hypothesize that there is no significant difference between the number of inflammatory cells and artifact found in the lung FNAB examination using cytology and cell block techniques. Therefore, this study analyzed the preservation of cytology and cell block smear specimens to prove that the cytology smear technique is of comparable quality to cell block technique in lung biopsy aspiration specimens using indicators of the number of inflammatory cells and the number of artifacts.

## METHODS

This study was an observational analytical study comparing the number and types of inflammatory cells in FNAB biopsy with cytology and cell block techniques. Sample population was the patients of Dr. Soetomo Hospital, Surabaya, Indonesia, suspected of lung cancer and underwent FNAB biopsy. Samples were taken with FNAB, where tissue or fluid samples were taken by aspiration using fine needle (no. 25). Thereafter, the sample was divided into two equal parts and processed

using cytology and cell block techniques.

Samples were obtained from the patients at Dr. Soetomo Hospital, Surabaya, who underwent CT guided-FNAB procedure. In this study, the results of FNAB procedure from one and the same individual were divided into two parts: one part was processed with cytology method and the other part was processed with cell block method. The initial sample size in this study was 38 pairs of slides.

The preparations obtained were observed under a microscope, then the number of inflammatory cells, consisting of neutrophils, lymphocytes, macrophages, and plasma cells, was counted quantitatively from one visual field. Thereafter, the results were converted into scores before being processed statistically so that the data can represent the entire visual field. The author also performed qualitative calculations of artifacts in all visual fields using a scoring system 0-3.

### Data Analysis for Inflammatory Cells

The number of inflammatory cells was observed microscopically in one visual field. In order to represent the entire visual field, the number of inflammatory cells were calculated quantitatively then converted into scoring system with this following criteria: (0). If  $x = 0$ ; (1). If  $x < \text{mean} - \frac{1}{2} \text{SD}$ ; (2). If  $\text{mean} - \frac{1}{2} \text{SD} \leq x < \text{mean} + \frac{1}{2} \text{SD}$ ; (3). If  $x \geq \text{mean} + \frac{1}{2} \text{SD}$ .

### Data Analysis for Artifacts

Each group had three artifact criteria. However, artifact criteria in cytology differed from artifacts in cell blocks. The types of artifacts in cytology were air bubbles, thick smear and crushing artifacts, while the types of artifacts in cell blocks included folded pieces, torn pieces and venetian blinds. The artifacts were observed in all visual field and calculated qualitatively using this following scoring criteria: (0). no artifacts found; (1). artifacts found < 10% of the entire visual field; (2). artifact with moderate finding (10-70% of the entire visual field); (3). artifact with high finding (>70% of the entire visual field).

### Comparative Test for Inflammatory Cells and Artifacts

We conducted a normality test regarding the number of inflammatory cells as well as the number of artifacts found. Normally distributed data were tested with comparison test of paired t-test, while data that were not normally distributed were tested with Wilcoxon-signed rank comparison test. Data of this study were processed statistically using SPSS and Microsoft Office Windows software.

## RESULTS

### Statistical Test for Inflammatory Cells Counts

Mean and standard deviation of each type of inflammatory cells and score can be seen in Table 1.

**Table 1. Mean and standard deviation of each type of inflammatory cells in one visual field**

Types of the inflammatory cells		Mean $\pm$ SD
<b>In one visual field:</b>		
Cytology	Neutrophil	6.74 $\pm$ 4.542
	Lymphocytes + Plasma	4.55 $\pm$ 13.268
	Macrophage	10.34 $\pm$ 18.533
	Neutrophil	37.05 $\pm$ 28.838

**Table 1. Mean and standard deviation of each type of inflammatory cells in one visual field (Continued)**

Types of the inflammatory cells		Mean ± SD
Cell Block	Lymphocytes + Plasma	13.97±14.744
	Macrophage	18.39±36.094
<b>In cytology and cell block:</b>		
Cytology	Neutrophil	1.87±0.844
	Lymphocytes + Plasma	1.39±1.054
	Macrophage	1.55±1.005
Cell Block	Neutrophil	1.82±0.982
	Lymphocytes + Plasma	1.68±0.989
	Macrophage	1.45±1.179

Scoring criteria for each type of inflammatory cells are listed in Table 2. Sample comparison test is needed to test whether there are significant differences between groups. In this study, the test was carried out using Wilcoxon-signed ranking test for abnormally distributed data and paired t-test for normally distributed data. The data had significant difference if the value of significance >0.05 (sig. >0.05). The results of sample comparison tests can be seen in Table 2. Sample comparison test showed that was no significant difference in the number of inflammatory cells score processed with cytology and cell block techniques.

**Table 2. Comparison test between inflammatory cells in cell block vs cytology**

Types of the inflammatory cells		Mean (in one visual field)	SD
Cytology	Neutrophil	6.74	4.542
	Lymphocytes + Plasma	4.55	13.268
	Macrophage	10.34	18.533
Cell Block	Neutrophil	37.05	28.838
	Lymphocytes + Plasma	13.97	14.744
	Macrophage	18.39	36.094

*Statistical Test of the Number of Artifacts*

The percentage of cytological artifacts found were air bubbles (41%), thick smears (33%) and crushing artifacts (26%), while the percentage of cell block artifacts were folded pieces (32%), torn pieces (45%) and venetian blinds (23%). The most cytological artifacts were air bubbles (41%), while the most cell block artifacts were torn pieces (45%).

The scores for each number of artifacts were totaled, then the average score between cytological artifacts and cell blocks was calculated. The higher the scoring results, the worse the quality of the preparations. Mean and standard deviation of total scores of cytological and cell block artifacts can be seen in Table 3.

There was a significant difference between the total score of cytology and cell block artifacts. Comparative tests showed significant differences between the total score of cytological and cell block artifacts.

**Table 3. Descriptive analysis of the total score of cytology and cell block artifacts**

Variables	%	Mean ± SD	P
<b>Total Score of Cytology Artifacts</b>			
Air bubble	41	4.52±1.536	0.027
Thick smear	33		
Crushing artefact	26		
<b>Total Score of Cell Block Artifacts</b>			
Folded pieces	32	3.85±1.477	
Torn pieces	45		
Venetian blind	23		

**DISCUSSION**

Cytology and cell block are specimen processing methods currently used in the practice of anatomic pathology. In this study, the authors used a scoring system in processing observational data due to specimens' quality between cytology and cell block. In cytology, the specimen obtained is processed to become thin and wide smear. While in cell blocks, specimen is compacted and immersed with paraffin blocks (6) so they cannot be counted and compared by just observing one field of view.

*Analysis of the Number of Inflammatory Cells*

Inflammatory cells comparison test results showed no significant difference between the number of inflammatory cells in cytology or cell block. This is different from previous studies which found that cell block had a higher cellularity than cytology (5,6). This was because the study did not observe the whole field of view and only observed one field of view. Specimens processed by cell block technique appear to have higher cellularity because in the cell block the specimen is compressed with paraffin blocks so that the cells are concentrated in one place. Then, it is found that cell block has another disadvantage. The specimen is cut with a microtome to a size of ±4-6µ (5), so the specimen becomes very small. Inflammatory cells and artifacts in the two samples obtained by the authors could not be evaluated because, due to the size of the specimen, the number was too small.

*Analysis of the Number of Artifacts*

Artifact is a structure that is not present in normal tissue and formed by tissue processing that can interfere with the process of cytopathological interpretation (8). Artifact can interfere with histopathological assessments by altering the appearance of a tissue, mimicking other tissues, or changing structures so that they are difficult to identify (9). On the results of artifacts comparison, the higher the score, the worse the preparation quality. Cytology artifacts use three criteria, i.e. air bubbles, thick smear and crushing artifacts. Whereas, cell blocks use three other criteria, i.e. folded pieces, torn pieces and venetian blinds. The maximum score of each inflammatory cell for artifacts is 9 (very poor quality) and a minimum of 0 (no artifacts at all). Based on statistical results, mean cytological artifacts score (4.52) was higher than mean cell block artifacts score (3.85).

Cytological artifact is defined as artificial structures or cellular changes due to external factors (8). In this study, it was found that cytology smear technique produced more artifacts than cell block. Other studies also obtained similar results, where the number of artifacts in cytology was higher than cell block (10-13). This was due to

inadequate processing techniques, including the process of specimen collection, preparation of smears, delayed fixation, and staining (14).

The most cytological artifact obtained is air bubbles. Air bubble artifacts are formed due to improper placement of the cover glass or the cover glass is placed when the preparation is still wet. In improper placement of the cover glass, some air bubbles are trapped in the preparation and, when staining is being performed, the part that contains the air bubbles is not adequately stained (15). In addition, air bubbles can also be formed in preparations that are stored too long due to imperfect dehydration, the type of mounting medium is the entellan which has a high reactivity index, dilution of the mounting medium uses too much xylene and causes the resin media to be too thin so that, when xylene evaporates, air space forms under the cover glass (16). Air bubble artifacts can be avoided by placing the cover glass at the right angle, adding water to the specimen before covering it with cover glass, using immersion oil, adding detergents to reduce surface tension, and soaking the specimen in alcohol to reduce water content (17). When air bubbles have formed on the specimen, there are several ways to overcome the problem, including heating and then tilting the preparation. If there are problems in specimen preparation due to xylene evaporation, the preparation can be dipped back into the xylene (18).

Cell block artifacts occur due to inappropriate specimen processing techniques. This technique has many stages so that the experience in specimen processing is required. Most cell block artifacts are torn pieces. This is because torn pieces can occur in the whole process, i.e. during the cutting, mounting, grossing, and processing, as well as improper attachment of the tissue. Blunt cutting blades, rough cutting, too soft embedding medium, poor flotation technique, careless removal of the specimen from the paraffin blocks, or poor specimen adhesion can cause specimens to be uplifted during staining (19).

This study found that there was no significant difference between the number of inflammatory cells in cytology and cell block specimens and that artifacts in cytological specimens can be solved so that cytology smear technique is still sufficiently standardized to establish the histopathological diagnosis. Our findings would suggest cell block technique is best used for immunohistochemistry or another further diagnosis rather than primary cytopathological diagnosis.

#### ACKNOWLEDGMENT

The authors thank the Faculty of Medicine, Universitas Airlangga and the Department of Anatomic Pathology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Hospital, who had facilitated this research.

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