Asia-Pacific Journal of Science and Technology: Volume: 27. Issue: 01. Article ID.: APST-27-01-11. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research and Technology Transfer Affairs Division,
Khon Kaen University, Thailand

Digital whole slide imaging for pleural fluid cytopathology diagnosis: Accuracy, sensitivity, specificity, and concordance

Raweewat Bannarakna[1], Sakda Waraasawapati[1], Tanin Titipungul[2], Nipon Chaisuriya[1] and Piyapharom Intarawichian[1,*]

[1]Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[2]Department of Pathology, Mahasarakham Hospital, Mahasarakham, Thailand
[*]Corresponding author: piyain@kku.ac.th

Received 7 May 2021
Revised 13 August 2021
Accepted 11 September 2021

Abstract

Digital whole slide imaging (WSI) is a developing technique enabling digital pathology for research applications. This investigation aimed to determine the accuracy, sensitivity, specificity, and concordance of non-gynecological cytopathology specimen (pleural fluid) diagnoses made using WSI and glass slides. Sixty pleural fluid cytology specimens were prepared in glass slides and scanned with a digital slide scanner for WSI. Two pathologists interpreted both the glass and digital slides then the accuracy, sensitivity, specificity, and concordance (Kappa coefficient) of their diagnoses were examined between the techniques. A three-week interval separated the WSI and glass slide interpretations. The results found by pathologists 1 and 2 showed that the WSI accuracy were 91% and 93%, sensitivity was 89.3% and 86.7%, specificity was 92.3% and 100%, and concordance (Kappa coefficients) were 87% (0.74, p <0.001) and 95% (0.90, p< 0.001), respectively. Therefore, WSI has the potential to be applied in the diagnosis of pleural fluid cytology with accuracy, sensitivity, specificity, and concordances equal to standard diagnosis techniques. In the future, the flexibility, and utility, and other potential aspects of WSI will be widely used in research and routine cytopathological diagnosis.

Keywords: Whole slide imaging, Digital pathology, Pleural fluid cytopathology, Diagnosis

1. Introduction

Optical microscopy is a conventional method that utilizes a light microscope to observe microscopic, pathological morphology and perform diagnoses in medicine. Regularly used from the past through today, it is a microscopy method employed in research, pathology, and laboratory medicine worldwide. Although optical microscopy is widely available, in many situations, it is limited. For example, optical microscopy often requires a workstation, and, in some problematic cases, expert pathologists are necessary for assessing certain features. Some of these conventional method weak points can be solved by digital imaging [1-4].

Digital imaging is becoming widely used by pathologists. Histopathology slides are each captured as a whole-slide image (WSI), also referred to as whole slide imaging, using a special whole-slide scanner. The digitized WSI can be further analyzed using computational approaches [5,6]. Nowadays, the use of WSI is widespread, with numerous applications in subspecialties of pathology [5-7]. Previous studies have shown that WSI is applied in surgical pathology areas, such as studies on frozen sections, diagnosis, and, through quantitative digital imaging analysis (DIA), on an interobserver agreement for categorical and quantitative liver fibrosis scores [8,9]. Furthermore, the WSI technique has been applied in gynecologic cytopathology with high accuracy. However, few studies have assessed the accuracy of WSI for non-gynecological cytopathology specimens [8-11].

Therefore, this study aimed to compare the accuracy, sensitivity, specificity, and concordance of the cytopathology of pleural fluid specimens from WSI with the conventional method of directly observing glass slides under a light microscope.

2. Materials and methods

2.1 Sample preparation

Sixty pleural fluid cytology slides were supplied by the Department of Pathology of Mahasarakham Hospital, Thailand. All samples were collected from the pleural fluid as a representative non-gynecological specimen and subjected to standard cytocentrifugation techniques. The selected slides were grouped into two categories: (1) negative for malignancy (30 slides) and (2) metastatic adenocarcinoma (30 slides). All slides were reviewed by two pathologists to confirm the diagnosis. In non-concordance, the cases and previous reports were reviewed, and a final diagnosis consensus was made.

2.2 Whole slide imaged scanning

All slides were scanned with an Aperio ScanScope slide scanner (ScanScope® XT, Leica Biosystems, IL, USA) at multiple focal planes, 40X optical magnification, and high resolution (54,000 pixels per inch). The images were saved as a .svs file. Each WSI was displayed with Aperio Image Scope program version 12.1.5029 (Leica Biosystems, IL, USA) running on a desktop computer and displayed on an HP monitor (resolution 1600x900) (Figure 1 (A)). The WSI quality was assessed into one of three categories: poor, fair, and good. Each WSI was interpreted by two WSI-trained pathologists (NC, PI), and each standard optical microscopy glass slide was interpreted by the same pathologists (Figure 1 (B)). All cases were assessed blindly, and the gap between WSI and glass slide interpretation was three weeks.

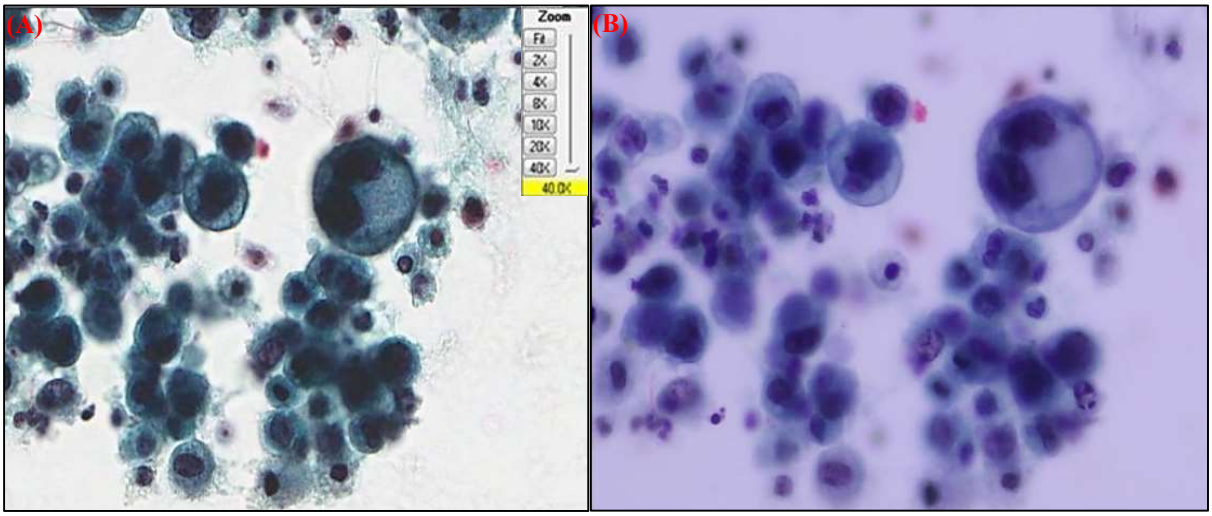

Figure 1 WSI displayed on an HP monitor (A) and photographed glass slide (B) in the same area (Pap stain, 400X).

2.3 Statistical analysis

For each of the two pathologists, diagnosis accuracy, sensitivity, specificity, and concordance were analyzed. Concordance was defined as the Kappa coefficient and categorized as identical, minor discordance, or major discordance. Minor discordance was described as a diagnosis difference that would not affect treatment and prognosis, while major discordance was described as a diagnosis difference that could affect treatment and prognosis. All parameters were analyzed for statistical significance by SPSS Statistics® version 18.0.

3. Results

The selected pleural fluid slides included categories of (1) negative for malignancy (30 slides) and (2) metastatic adenocarcinoma (30 slides). All samples were collected from pleural fluid, subjected to standard cytocentrifugation techniques, and analyzed by two pathologists. Pathologist 1 presented accuracy for glass slides (conventional microscopy) and WSI of 93% and 91%, respectively, while pathologist 2 found accuracy for glass slides and WSI to be 95% and 93%, respectively (Table 1).

Table 1 The accuracy of glass slides and WSI by two pathologists.

Method	Malignant	Pathologist 1				Pathologist 2			
		Same Diagnosis			Accuracy (%) [95%CI]	Same Diagnosis			Accuracy (%) [95%CI]
		No	Yes	n		No	Yes	n	
Glass slides	No	30	4	34	56/60, 93% [83.8- 98.2]	29	2	31	57/60, 95% [86.1-99.0]
	Yes	0	26	26		1	28	29	
	n	30	30			30	30		
WSI	No	24	3	27	49/54, 91% [79.7 - 96.9]	30	4	34	56/60, 93% [83.8-98.2]
	Yes	2	25	27		0	26	26	
	n	26	28			30	30		

To compare the diagnosis performance of glass slides to WSI, sensitivity and specificity were calculated. As shown in Table 2, pathologist 1 found sensitivity and specificity of 86.7% and 100%, respectively, for glass slides and 89.3% and 92.3%, respectively, for WSI, while pathologist 2 found 93.3% and 96.7% for glass slides and 86.7% and 100% for WSI. For each pathologist, the difference in performance between glass slides and WSI was unremarkable.

Table 2 The sensitivity and specificity of glass slides and WSI by two pathologists.

Method	Sensitivity		Specificity	
	%	95% CI	%	95% CI
Pathologist 1				
Glass slides	86.7	[69.3-96.2]	100.0	[88.4-100.0]
WSI	89.3	[71.8-97.7]	92.3	[74.9 -99.1]
Pathologist 2				
Glass slides	93.3	[77.9-99.2]	96.7	[82.8-99.9]
WSI	86.7	69.28-96.24	100.0	[88.4-100.0]

Kappa coefficients were established to determine the concordance. Pathologist 1 defined the concordance as 87% or 0.74 (p <0.001) while pathologist 2 defined it as 95% or 0.90 (p <0.001) (Table 3). In addition, the major discordance of pathologists 1 and 2 were 13% and 3%, respectively (Table 4). The estimation summary of the WSI-based diagnosis from the two pathologists is shown in Table 4.

Table 3 The concordance of glass slides and WSI diagnosis by two pathologists.

Method	Malignant	Pathologist 1					Pathologist 2				
		Glass slides			Concordance	Kappa coefficient (p-value)	Glass slides			Concordance	Kappa coefficient (p-value)
		No	Yes	n			No	Yes	n		
WSI	No	25	2	27	87%	0.74	31	3	34	95%	0.90
	Yes	5	22	27		(p<0.001)	0	26	26		(p<0.001)

Table 4 The summary of accuracy and concordance in WSI by two pathologists**.**

Parameter	Pathologist 1	Pathologist 2
Accuracy of WSI (%)	91	93
Kappa coefficient of WSI with glass slide (Concordance)	0.74 (p<0.001)	0.90 (p<0.001)
Major discordance of WSI with glass slide (%)	13	3

The average interpretation time for WSI was longer than for glass slides. The percentage of cases that took less than one minute to interpret for glass slides and WSI were 80% and 50%, respectively. The percentage of cases that took between one to two minutes to interpret for glass slides and WSI were 18% and 37%, respectively, and the percentage of cases that took greater than two minutes to interpret for glass slides and WSI were 2% and 13%, respectively (Figure 2 (A)).

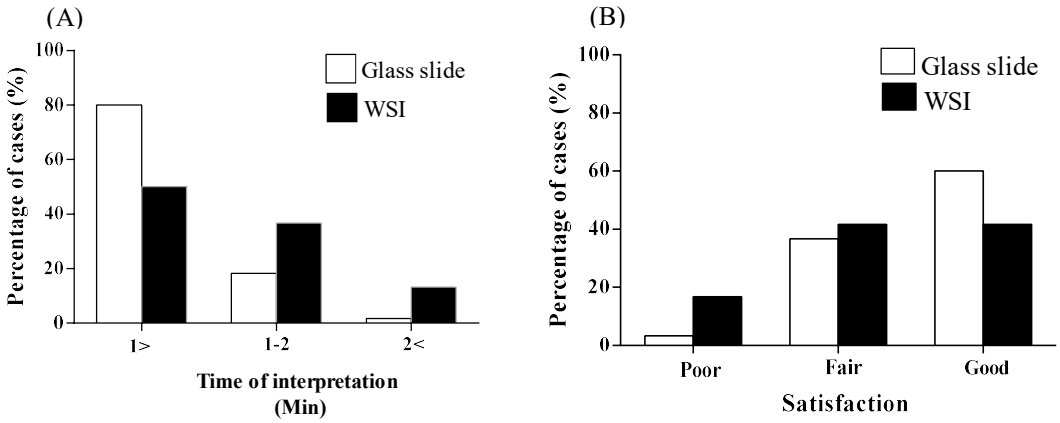

Figure 2 The comparison time of interpretation between the glass slide and WSI (A) and the comparison time of satisfaction between glass slides and WSI (B).

Finally, questionnaires were assigned to the two pathologists to evaluate the completed structure observed in WSI compared to the glass slides. The quality satisfaction results were 3% and 16% poor, 37% and 42% fair, and 60% and 42% good for glass slides and WSI, respectively (Figure 2 (B)).

The two pathologists voted that some advantages of WSI were: (1) convenient to view images in laptops and mobile devices, (2) easy to capture images, (3) slide colors do not fade, (4) convenient to transfer files to other pathologists and (5) easy to duplicate the WSI for sharing. The two pathologists complained that some disadvantages of WSI were: (1) not easy to move and pan the image across the screen, (2) difficult to see cytomorphologic details in a thick area and (3) the size of the file is too large.

4. Discussion

Our results were consistent with previous studies, which reported a 75-90% concordance and an accuracy of 77-90% [12-14]. The slide scanner has a multiple focal plane capture function, which can automatically focus across multiple layers of interesting areas. This function produces a cytopathology multilayer and increases accuracy and concordance. David M et al. reported that WSI could be used in non-gynecological cytopathology [15]. In addition, [16] compared the potential of diagnostic concordance between WSI and conventional light microscopy in cytopathology using a liquid-based cytology cervical specimen. The result revealed that concordance between WSI and traditional light microscopy was 92%, with a Kappa coefficient of 0.66 and an interobserver Kappa coefficient of 0.69. The time to reach a diagnosis was longer with WSI in all studies [16]. Similarly, our study showed that WSI provided high concordance (concordance = 87% and 95% and Kappa coefficient = 0.74 and 0.90), accuracy (91% and 93%), sensitivity (89.3% and 86.7%), and specificity (92.3% and 100%) for the two pathologists in the cytopathology of pleural fluid.

Advantages of WSI over glass slides are that the stained color does not fade over time, and the image file can be duplicated easily for sharing. A disadvantage of WSI vs. conventional diagnosis is the longer interpretation time mentioned, potentially because WSI requires a computer and mouse for image panning, making the viewing physically uncomfortable. The major discordance observed in our study might be explained by the familiarization of WSI and the experience of each pathologist.

Nowadays, WSI technology development supports increasingly high-performance image capture and quality, storage, and vision software. WSI has a significant influence on cytopathology in many applications, such as cytology quality assurance, education, and trainee efficacy assessment [3,6,17,18]. Innovation and technology familiarization will promote performance and make digital whole-slide review feasible for larger telepathology subspecialty consultation applications [19-21]. We suggest that pathologists should ensure they familiarize themselves with the available WSI viewer tools, e.g., thumbnails, auto panning, keyboard navigation, and tracker capabilities [22]. Further studies should incorporate a larger sample size, other non-gynecologic specimen types, and multicenter research.

This study was limited in multiple aspects. First, the non-gynecologic specimens consisted only of pleural fluid. Additional non-gynecological cytopathology, such as cerebrospinal, pericardial, synovial, and gastrointestinal fluid, should also be examined to help further validate the accuracy, sensitivity, specificity, and concordance in WSI performance. Second, this study was performed by only two investigators (referred to throughout this study as pathologists 1 and 2). More than two pathologists are needed to compare the diagnostic performance between the glass slide and WSI more accurately.

In a prospective study, WSI development will have continued and lead to even higher-resolution images for poor-quality samples [6,18]. Moreover, we plan to develop WSI with machine learning by artificial intelligence (AI) to assist pathological diagnosis. Therefore, this study is early in developing AI-supported disease diagnosis in medicine [23-27].

5. Conclusion

WSI has a high accuracy and concordance in the diagnosis of cytopathology. In future research, WSI will be applied in a significant role in the non-gynecological cytopathology field.

6. Ethical approval

The study was conducted according to approval by the Ethics Committee of Khon Kaen University (HE591041, date of approval 26 January 2016).

7. Acknowledgments

The authors would like to acknowledge Mr. Kevin McCracken for the English language editing of this manuscript via the KKU Publication Clinic (Thailand).

8. References

[1] Cunningham CM, Larzelere ED, Arar I. Conventional microscopy vs. computer imagery in chiropractic education. J Chiropr Educ. 2008;22(2):138-144.

[2] Weinstein RS, Graham, AR, Richter LC, Barker GP, Krupinski EA, Lopez AM, et al. Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future. Hum Pathol. 2009; 40(8):1057-1069.

[3] Higgins C. Applications and challenges of digital pathology and whole slide imaging. Biotech Histochem. 2015;90(5):341-347.

[4] Hanna MG, Monaco SE, Cuda J, Xing J, Ahmed I, Pantanowitz L. Comparison of glass slides and various digital-slide modalities for cytopathology screening and interpretation. Cancer Cytopathol. 2017; 125(9):701-709.

[5] Wright AM, Smith D, Dhurandhar B, Fairley T, Scheiber PM, Chakraborty S, et al. Digital slide imaging in cervicovaginal cytology: a pilot study. Arch Pathol Lab Med. 2013;137(5):618-624.

[6] Chantziantoniou N, Mukherjee M, Donnelly AD, Pantanowitz L, Austin RM. Digital applications in cytopathology: problems, rationalizations, and alternative approaches. Acta Cytol. 2018;62(1):68-76.

[7] Nishat R, Ramachandra S, Behura SS, Kumar H. Digital cytopathology. J Oral Maxillofac Pathol. 2017; 21(1):99-106.

[8] Steinberg DM, Ali SZ. Application of virtual microscopy in clinical cytopathology. Diagn Cytopathol. 2001;25(6):389-396.

[9] Pavlides M, Birks J, Fryer E, Delaney D, Sarania N, Banerjee R, et al. Interobserver variability in histologic evaluation of liver fibrosis using categorical and quantitative scores. Am J Clin Pathol. 2017; 147(4):364-369.

[10] Marchevsky AM, WanY, Thomas P, Krishnan L, Evans SH, Haber H. Virtual microscopy as a tool for proficiency testing in cytopathology: a model using multiple digital images of papanicolaou tests. Arch Pathol Lab Med. 2003;127(10):1320-1324.

[11] Van SL, Kumar RK, Pryor WM, Salisbury EL, Velan GM. Cytopathology whole slide images and adaptive tutorials for senior medical students: a randomized crossover trial. Diagn Pathol. 2016;11(1):1-9.

[12] Pantanowitz L, Sinard JH, Henricks WH, Fatheree LA, Carter AB, Contis L, et al. Validating whole slide imaging for diagnostic purposes in pathology: guideline from the college of American pathologists pathology and laboratory quality center. Arch Pathol Lab Med. 2013;137(12):1710-1722.

[13] Mukhopadhyay S, Feldma MD, Abels E, Ashfaq R, Beltaifa S, Cacciabeve NG, et al. Whole slide imaging versus microscopy for primary diagnosis in surgical pathology: a multicenter blinded randomized noninferiority study of 1992 cases (pivotal study). Am J Surg Pathol. 2018;42(1):39-52.

[14] Hanna MG, Reuter VE, Hameed MR, Tan LK, Chiang S, Sigel C, et al. Whole slide imaging equivalency and efficiency study: experience at a large academic center. Mod Pathol. 2019; 32(7):916-928.

[15] Wilbur DC, Madi K, Colvin RB, Duncan LM, Faquin WC, Ferry JA, et al. Whole-slide imaging digital pathology as a platform for teleconsultation: a pilot study using paired subspecialist correlations. Arch Pathol Lab Med. 2009;133(12):1949-1953.
[16] Girolami I, Pantanowitz L, Marletta S, Brunelli M, Mescoli C, Parisi A, et al. Diagnostic concordance between whole slide imaging and conventional light microscopy in cytopathology: a systematic review. Cancer Cytopathol. 2020;128(1):17-28.
[17] Saco A, Bombi JA, Garcian A, Ramirez J, Ordi J. Current status of whole-slide imaging in education. Pathobiology. 2016;83(2-3):79-88.
[18] Kumar N, Gupta R, Gupta S. Whole slide imaging (WSI) in pathology: current perspectives and future directions. J Digit Imaging. 2020;33(4):1034-1040.
[19] Farris AB, Cohen C, Rogers TE, Smith GH. Whole slide imaging for analytical anatomic pathology and telepathology: practical applications today, promises, and perils. Arch Pathol Lab Med. 2017; 141(4):542-550.
[20] Bashshur RL, Krupinski EA, Weinstein RS, Dunn MR, Bashshur N. The empirical foundations of telepathology: evidence of feasibility and intermediate effects. Telemed J E Health. 2017;23(3):155-191.
[21] Alami H, Fortin JP, Gagnon MP, Pollender H, Tetu B, Tanguay F. The Challenges of a complex and innovative telehealth project: a qualitative evaluation of the Eastern Quebec telepathology network. Int J Health Policy Manag. 2018;7(5):421-432.
[22] Khalbuss WE, Cuda J, Cucoranu IC. Screening and dotting virtual slides: a new challenge for cytotechnologists. Cytojournal. 2013;10(1):22.
[23] Shen J, Zhang JP, Jiang B, Chen J, Song J, Liu Z, et al. Artificial intelligence versus clinicians in disease diagnosis: systematic review. JMIR Med Inform. 2019;7(3):e10010.
[24] Bi WL, Hosny A, Schabath MB, Giger ML, Birkbak NJ, Mehrtash A, et al. Artificial intelligence in cancer imaging: clinical challenges and applications. CA Cancer J Clin. 2019;69(2):127-157.
[25] Wang S, Yang DM, Rong R, Zhan X, Fujimoto J, Liu H, et al. Artificial intelligence in lung cancer pathology image analysis. Cancers (Basel). 2019;11(11):1673.
[26] Park SH, Han K. Methodologic guide for evaluating clinical performance and effect of artificial intelligence technology for medical diagnosis and prediction. Radiology. 2018;286(3):800-809.
[27] Bera K, Schalper KA, Rimm DL, Velcheti V, Madabhushi A. Artificial intelligence in digital pathology - new tools for diagnosis and precision oncology. Nat Rev Clin Oncol. 2019;16(11):703-715.