Digestive and Liver Disease 51 (2019) 1574–1579

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

Concordance, intra- and inter-observer agreements between light microscopy and whole slide imaging for samples acquired by EUS in pancreatic solid lesions

Alberto Larghi [a], Adele Fornelli [b,*], Stefania Lega [c], Moira Ragazzi [d], Gabriele Carlinfante [d], Paola Baccarini [e], Carlo Fabbri [f], Paola Pierotti [b], Giovanni Tallini [g], Arrigo Bondi [b], Dario de Biase [h]

[a] Digestive Endoscopy Unit, Foundation University Hospital Policlinico A. Gemelli IRCCS, CERTT, Center for Endoscopic Research Therapeutics and Training, Catholic University, Rome, Italy
[b] Pathology Unit, Maggiore Hospital, Bologna, Italy
[c] Pathology Unit, Ospedale Vecchio, Imola, Italy
[d] Pathology Unit, S. Maria Nuova Hospital, IRCSS-AUSL Reggio Emilia, Italy
[e] Pathology Unit, Bellaria Hospital, Bologna, Italy
[f] Digestive Endoscopy and Gastroenterology, Forlì and Cesena Hospitals, Italy
[g] Anatomical Pathology, Molecular Diagnostic Unit, University of Bologna School of Medicine, Bologna, Italy
[h] Department of Pharmacy and Biotechnology, Molecular Diagnostic Unit, University of Bologna, Bologna, Italy

ARTICLE INFO

Article history:
Received 10 March 2019
Accepted 29 April 2019
Available online 28 May 2019

Keywords:
Endoscopic ultrasound, fine needle biopsy
Light microscopy
Pancreatic cancer
Whole slide imaging

ABSTRACT

Background: No study has compared the performance of light microscopy (LM) and whole slide imaging (WSI) for endoscopic ultrasound (EUS) histological acquired tissue samples from pancreatic solid lesions (PSLs). We evaluated the concordance between LM and WSI and the inter- and intra-observer agreements among pathologists on PSLs EUS acquired samples.
Methods: LM and WSI from 60 patients with PSLs were evaluated by five expert pathologists to define: diagnostic classification, presence of a core, number and percentage of lesional cells. Washout period between evaluations was 3 months. Time of the procedures was also assessed.
Results: Forty-eight cell-block and 12 biopsy samples were evaluated. A high concordance between LM and WSI was found. Inter- and intra-observer agreements for diagnostic classification were substantial and complete, respectively. For all the other parameters, the inter-observer agreement was usually higher for LM. For the intra-observer, a substantial agreement was reached regarding the presence of tissue core and the number and the percentage of malignant cells. Median time for performing LM was significantly shorter than for WSI ($p < 0.0001$).
Conclusions: LM and WSI of cell-block and biopsy samples acquired by EUS in PSLs were highly concordant, with a substantial inter-observer and a complete intra-observer agreements regarding diagnostic classification.

1. Introduction

Pathological diagnosis of many diseases has been traditionally obtained by using light microscopy (LM). In 1986, digital pathology was introduced under the concept of "telepathology" that included two forms, the dynamic (real-time) robotic "telepathology" and the static image (store-and-forward) "telepathology" [1]. Subsequently, various technologies were developed, until the introduction of whole slide imaging (WSI) that represents the latest innovation utilized in digital pathology [2,3].

With WSI, also commonly referred as to "virtual microscopy", conventional glass slides are scanned in order to produce digital slides. At present, the main utilization of WSI is teleconsultation (i.e. diagnostic evaluation from remote computers), but its value in educational and research pathology is exponentially growing [4–6]. WSI can also become useful for the process of standardization of biomarkers in tissues, as highly recommended by the US Food and

* Corresponding author at: Pathology Unit, Azienda USL Città di Bologna, Ospedale Maggiore, Largo Nigrisoli, 2, 40133, Bologna, Italy.
E-mail address: adele.fornelli@ausl.bologna.it (A. Fornelli).

https://doi.org/10.1016/j.dld.2019.04.019
1590-8658/

Drug Administration (FDA) and the American Society of Clinical Oncology, and the College of Pathologists [7,8].

A number of studies have reported a very good correlation between standard glass slides and WSI for both routine and consultative surgical pathology cases in breast [9,10], gastroenterology [11–13], prostate [14,15], pulmonary [16], skin [17], and mixed-specimen biopsies [18,19]. A large multicenter randomized non-inferiority study on 1992 cases concluded that WSI is not inferior to LM for primary diagnosis concerning biopsies and resection stained H&E, immunohistochemistry and special stains [20]. In addition, utilization of WSI can significantly save money, reduce turnaround time and facilitate the exchange of information [21]. This growing support of digital pathology culminated in the April 2017 announcement by the FDA approving the use of WSI for primary diagnosis in surgical pathology [22,23].

Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has become an irreplaceable tool in the diagnostic and staging algorithm of lesions of the gastrointestinal tract or adjacent structures [24]. Diagnostic accuracy, however, strongly depends on rapid on-site evaluation (ROSE), which determines a significant 10%–15% gain [25]. Nevertheless, ROSE is not available in many centers because of costs, physicians' availability and expertise [26]. Attempts to use telepathology instead of ROSE have been successfully documented [27,28], but this technology has not gained widespread use. In addition, the development of new needles for EUS-guided fine needle biopsy (EUS-FNB) able to reach an accuracy equal or similar to that of EUS-FNA with ROSE [29], is determining a moving from cytology to histology in this field [30].

Up to now, no study has evaluated the concordance between LM and WSI and the intra- and inter-observer agreements between pathologists on histological samples from pancreatic solid lesions acquired under EUS guidance.

2. Material and methods

Archival glass slides from 60 patients (M/F, 28/32) with pancreatic solid lesions were retrieved from the files of the Department of Pathology of the Maggiore Hospital in Bologna, Italy.

A representative number of cases per each diagnostic category, according to the Papanicolau Society of Cytopathology System for Reporting Pancreatobiliary Cytology [31], was selected. All samples, which were obtained under EUS-guidance using needles for FNA or FNB, were placed in formalin for cell-block preparation or for direct examination as a conventional endoscopic biopsy when macroscopically whitish tissue fragments could be visualized in the collecting vial. All samples were embedded in paraffin and subsequently stained with Hematoxylin and Eosin.

The samples were anonymized and identified by serial numbers. Clinical and anagraphic data were made usable to all pathologists involved in the study and a self-constructed online questionnaire was submitted to them to report their evaluations.

All H&E samples and scanned images were independently reviewed by five expert pathologists from three different centers (Department of Pathology, Maggiore Hospital, Bologna; Department of Pathology, Bellaria Hospital, Bologna; Department of Pathology, IRCCS Santa Maria Nuova, Reggio Emilia) using both LM and WSI. The evaluation of digital slides was performed after a washout period of three months from the LM reading.

The study was submitted to each hospital Research Ethics Board. The Boards determined that this was a quality assurance review that did not constitute human subject research, and, therefore, the requirement for approval from the board was waived. All samples will be treated in anonymous way and according to Declaration of Helsinki.

2.1. Histopathological criteria

The following criteria were evaluated:

1 Diagnostic classification, according to the Papanicolau Society of Cytopathology System for Reporting Pancreatobiliary Cytology: (I) Non-diagnostic; (II) Negative (for malignancy); (III) Atypical; (IV) Neoplastic (benign, other); (V) Suspicious for malignancy; (VI) Positive or Malignant.
2 Presence of a tissue core, defined as an architecturally intact piece of tissue measuring at least 550 μm in greatest axis corresponding approximately to the diameter of a high-power microscopic field [32].
3 Number of lesional cells: ≥500, <500, not evaluable.
4 Percentage of lesional cells: (i) >70%; (ii) 51–70%; (iii) 21–50%; (iv) 10–20%; (v) <10%; (vi) not evaluable.

Time to perform the diagnosis was calculated using a handheld digital timer as follow: (i) for LM the timer was started when the pathologist placed the slide on the microscope's stage and stopped at the moment when the diagnosis was reached; (ii) for WSI, the timer was started when the pathologist clicked on the file case to open the digital image and stopped at the moment of the diagnosis.

2.2. Slides preparation and digital scan

One slide per case was selected for the purposes of the present study. All 60 slides were digitized using an Aperio ScanScope XT scanner (Leica-Biosystems, Buffalo Grove, IL) at 20 × magnification (0.50 μm/pixel). The digital images were then loaded on the Aperio server and evaluated using the Aperio ImageScope (Leica-Biosystems, Buffalo Grove, IL) software.

At each site, pathologists used their own optical microscope for LM evaluation and their own personal computer (Windows Operating System, Intel Pentium Dual Core/Intel Core i3 processors, 3GB RAM memory, GoogleChrome/Firefox/InternetExplorer Browsers, 21″ widescreen monitors), and the network available in their respective working Institutions for digital evaluation.

Definitive diagnoses reached at Maggiore Hospital were considered the gold standard and were used to perform the comparison between LM and WSI and evaluation of the inter- and intra-observer agreements.

2.3. Statistical analysis

Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated in comparison to the definitive diagnoses.

Free-marginal multirater kappa was used to evaluate inter-observer agreement between LM and WSI images, while the intra-observer agreement was calculated using the Cohen kappa statistic. For ordinal categories, the weighted Kappa was calculated. Free-Marginal Multirater Kappa was used to evaluate interobserver agreement. Free-marginal multirater kappa and Cohen kappa were graded as: complete agreement ($\kappa > 0.81$), substantial agreement ($\kappa = 0.61–0.80$), moderate agreement ($\kappa = 0.41–0.60$), fair agreement ($\kappa = 0.21–0.40$) or slight agreement ($\kappa = 0.01–0.20$). Time for performing diagnosis was compared using the Wilcoxon matched-pairs signed rank test. A p-value < 0.05 was considered statistically significant.

3. Results

Overall, among the 60 specimens retrieved for the study, 48 cases were processed as cell-blocks, while the other 12 were treated

Table 1
Performance of light microscope and whole slide imaging in the evaluation of samples acquired by endoscopic ultrasound in 60 patients with pancreatic solid lesions.

	Light microscopy [95% CI]	Whole slide imaging [95% CI]	p Value
Sensitivity	0.92 [0.87–0.95]	0.93 [0.89–0.95]	NS
Specificity	0.96 [0.80–0.99]	0.88 [0.69–0.97]	NS
Positive predictive value	0.99 [0.97–0.99]	0.99 [0.97–0.99]	NS
Negative predictive value	0.51 [0.41–0.61]	0.52 [0.41–0.63]	NS
Diagnostic accuracy	0.92 [0.88–0.94]	0.92 [0.88–0.94]	NS

CI, Confidence Interval; NS: non statistically significant.

Table 2
Interobserver agreement on 60 slides from patients with solid pancreatic lesions evaluated by the five pathologists using light microscopy and whole slide imaging.

	light microscopy percent overall agreement [κ; 95% CI]	Whole slide imaging percent overall agreement [κ; 95% CI]	p Value
Diagnostic classification	84.5% [0.79; 0.71–0.88]	83.5% [0.78; 0.69–0.87]	NS
Presence of core tissue	79.3% [0.59; 0.45–0.72]	76.3% [0.53; 0.40–0.66]	NS
Number of lesional Cells	74.3% [0.62; 0.52–0.71]	68.7% [0.53; 0.43–0.63]	NS
Percentage of lesional Cells[a]	50.2% [0.40; 0.30–0.50] (78.3% [0.67; 0.57–0.78])	50.2% [0.38; 0.28–0.47] (77.8% [0.67; 0.57–0.77])	NS

K, Free-Marginal Multirater Kappa; CI, Confidence Interval; NS, non-statistically significant.
[a] Percent of lesional cells was calculated utilized all categories (<10%, between 10% and 20%, between 21% and 50%, between 51% and 70%, >70%) or assembling all categories in two groups < 50% or >50%.

as conventional endoscopic histological core biopsy samples. The definitive diagnoses of patients involved in the study based on the reading of the above slides at Maggiore Hospital were: 32 adenocarcinomas, nine neuroendocrine neoplasms, five metastatic neoplasia, three chronic pancreatitides, two lymphomas, one solid pseudopapillary tumor, one gastrointestinal stromal tumor, one accessory spleen. Moreover, there were three additional cases that were suspicious for malignancy and other three in which the sample was judged to be inadequate to make a definitive diagnosis.

3.1. Comparison between Light Microscopy and whole slide imaging

The overall performance of both techniques in the evaluation of the 60 specimens is shown in Table 1. Equal or very similar sensitivity, specificity, PPV, NPV, and diagnostic accuracy were observed between the two techniques. Misinterpretation by at least 3 pathologists was observed in eight cases (six adenocarcinomas and two neuroendocrine neoplasms).

3.2. Interobserver Agreement between light microscope and whole slide imaging

Inter-observer agreement between LM and WSI on histopathological parameters is shown in Table 2. A substantial inter-observer agreement as regard to the diagnostic classification was observed using both LM (84.5%, κ = 0.79, 95% C.I. 0.71–0.88) and WSI (83.5%; κ = 0.78, 95% C.I. 0.69–0.87). Regarding all the other parameters, the inter-observer agreement was usually higher, but not statistically significant, for LM (Table 2). Interestingly, inter-observer agreement on the "percentage of lesional cells" was only fair using both LM and WSI (κ = 0.40 and κ = 0.38, respectively), while a substantial agreement (κ = 0.67) was reached when the number of lesional cells (more or less than 500) was evaluated (Table 2).

3.3. Intra-observer agreement between light microscope and whole slide imaging

The intra-observer agreement between LM and WSI on histopathological parameters is shown in Table 3. A complete agreement (κ = 0.87, 95% C.I. 0.81–0.93) between LM and WSI was reached for the diagnostic classification. When using WSI, 22 of 300 (7.3%) of the slides were underestimated (i.e. the pathologist evaluated a lesion as "less malignant" with WSI than LM) and 32/300 (10.7%) were overestimated (i.e. pathologist evaluated a lesion as "more malignant" with WSI than with LM). A substantial agreement (κ = 0.68, 95% C.I. 0.59–0.77) was reached between LM and WSI regarding the presence of tissue core biopsy specimens. In particular, when using WSI 15 of 300 (5.0%) of the slides were underestimated and 29 of 300 (9.7%) were overestimated.

Regarding the evaluation of the number of lesional cells, a substantial agreement (κ = 0.67, 95% C.I. 0.56–0.77) was reached between LM and WSI. When using WSI, 23 of 300 (7.7%) of the slides were underestimated and 56 of 300 (18.7%) were overestimated. Similarly, to the number of cells, a substantial agreement (κ = 0.77, 95% C.I. 0.71–0.83; Table 3) was reached between LM and WSI also for the percentage of lesional cells. When using WSI, 59 of 300 (19.7%) of the slides were underestimated and 60 of 300 (20%) were overestimated.

The median time needed for performing the evaluation of slides using LM was 84 s (range 30–150), which was significantly shorter than the time needed to perform the evaluation with WSI [(108 s (range 54–240); p < 0.0001] (Fig. 1).

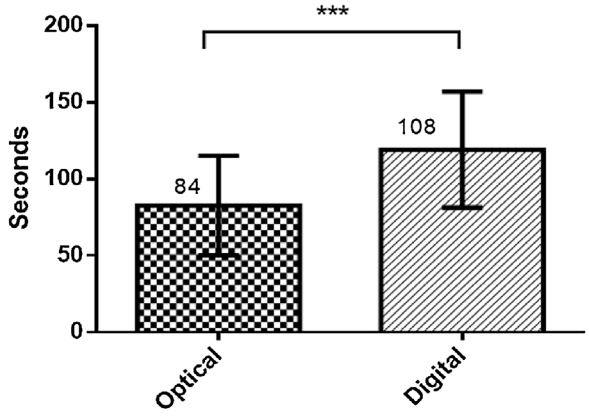

Fig. 1. Comparison of time for performing diagnosis using light microscopy or whole digital images. Vertical bars mean minimum and maximum time. ***p < 0.0001 (Wilcoxon matched-pairs signed rank test).

Table 3
Intra-observer agreement on 60 slides from patients with solid pancreatic lesions evaluated by the five pathologists using light microscopy and whole slide imaging.

Scored variables	Light microscopy (N = 300)[a]	Digital slide (N = 300)[a]	Agreement κ
Diagnostic classification			
I – Non-diagnostic	7.3%	6.3%	
II – Negative (for malignancy)	6.3%	5.6%	
III – Atypical	3.7%	5.0%	0.87
IV – Neoplastic (benign, other)	20.7%	18.7%	
V – Suspicious (for malignancy)	8.0%	6.7%	
VI – Positive or malignant	54.0%	57.7%	
Presence of core tissue			
Yes	61.0%	67.0%	0.68
No	39.0%	33.0%	
Number of lesional cells			
>500	54.3%	60.0%	0.67
<500	34.0%	30.3%	
N/A	11.7%	9.7%	
Percentage of lesional cells			
>70%	49.7%	50.7%	
51–70%	15.0	17.7%	
21–50%	11.3%	9.7%	0.77
10–20%	6.0%	5.0%	
<10%	6.0%	5.3%	
N/A	12.0%	11.7%	

[a] N = 300: 60 slides × 5 pathologists; N/A: not applicable.

4. Discussion

We performed a retrospective study aimed at evaluating the concordance and the inter- and intra-observer agreements between light microscopy and whole slide imaging among expert pathologists in the evaluation of cell-block and histological tissue core biopsy specimens acquired under EUS guidance in 60 patients with solid pancreatic lesions. Overall, we found a high concordance between LM and WSI, a substantial inter-observer agreement and a complete intra-observer agreement regarding diagnostic classification, which was the most important parameter evaluated.

Whole slide imaging, also commonly called virtual microscopy, refers to scanning of conventional glass slides in order to produce digital slides. It has become an increasingly important and exponentially utilized tool in telepathology for primary diagnosis, consultation (second opinion), and remote evaluation of frozen sections [4–6]. Additional uses comprise: (i) education, with the possibility to show images at tumor boards and other clinical conferences or to be able to give trainees the possibility to look at WSI slides from any computer, from any place ;[5] and (ii) research, with the capability for example of easily sharing a high number of slides for multicenter studies, thus avoiding time and money consuming process of mailing them to different centers for LM evaluation, which can prevent the good success of the study [33]. Similarly, pathology and biomarker communities can utilize WSI to: (i) create image repositories, which can be helpful to validate virtual slides collections; (ii) evaluate studies on concordance; (iii) develop an algorithm of image analysis [34]. Finally, WSI can also become useful for the process of standardization of tissue biomarkers, as highly recommended by the US FDA and the American Society of Clinical Oncology, and the American College of Pathologists [7,8].

In our study, we found a high concordance between LM and WSI, with a diagnostic accuracy of 92% for both techniques. Similarly, a recent systematic review of the literature on 32 studies, evaluating different organs, has found a concordance of 92.4% [35]. Moreover, a large multicenter randomized non-inferiority study on 1992 cases has concluded that WSI is not inferior to LM for primary diagnosis [20]. Importantly, our study is one of the few reports that have evaluated histological non-surgical samples. Indeed, differently from two previous experiences that assessed the value of telecytopathology for pancreatic cytological samples gathered by EUS-FNA, we evaluated cell-block and histological core biopsy samples. This is important because a shift from cytology to histology is rapidly occurring in the field of EUS-guided tissue acquisition, mainly driven by the availability of newly designed highly performing FNB needles with a diagnostic accuracy over 95%, and by the need to acquire histological samples to pave the road of personalized medicine [29,30]. EUS-FNB biopsies can be processed both as cell-block or histological samples depending on the center protocol [29,32]. It is possible that the lack of cytological samples may have contributed to our very satisfactory results, thus avoiding the underperformance of WSI when scanning thick cytological smears or specimens that have three-dimensional cell groups [36,37]. In the future, it is plausible to hypothesize that telepathology for EUS guided acquired pancreatic samples can be performed either at the time of the procedure, with preparation of a cytological smear using the touch imprint cytology technique from an FNB needle, which has been demonstrated to be equal than a smear obtained with a needle for FNA [38], or one day after when the specimen has been processed as a histological sample. In the first case scenario, at least a cytotechnician has to be involved in the procedure or endoscopists will have to learn how to make their own slides and instantaneous pictures of the most adequate material from a microscope to send them to a remote cytopathologist. Moreover, WSI of histological samples can be helpful in developing studies involving immunostaining evaluation, such as SMAD4 evaluation on EUS-FNB samples, which has a prognostic significance [39]. Future studies are needed to better clarify the time and economic impacts of these two strategies on EUS-guided tissue acquisition of pancreatic and non-pancreatic lesions.

A substantial inter-observer and a complete intra-observer agreement as regard to the diagnostic classification of samples were observed using both LM and WSI. These results were obtained after a washout period of three months, which is far superior to the period of at least two weeks recommended by the Guidelines from the College of American Pathologists Pathology and Laboratory Quality Center [40]. Regarding all the other parameters evaluated, in the inter-observer agreement LM had a better performance than WSI. Interestingly, the inter-observer agreement on the "percentage of lesional cells" was only fair using both LM and WSI. This result may reflect the poor habit of pathologists in the evaluation of the percentage of this parameter on histological samples from patients with solid pancreatic lesions. Indeed, the concordance grew up to a substantial agreement when the evaluation was done only based on the number of cells using a single cut-off (<500; ≥500).

In addition to the complete intra-observer agreement as regard to the diagnostic classification, a substantial agreement was also found considering the presence of tissue core biopsy specimens and of the percentage and number of lesional cells. Because of the growing evidence of the upcoming need to perform such analyses on pancreatic biopsy specimens to perform individualized therapies [41], guidelines and training to overcome this limitation should be organized.

The median time needed to perform WSI evaluation was significantly longer than the one occurred to examine LM slides ($p < 0.0001$). This result is in accordance with three previous studies all showing an advantage of LM regarding the time spent to review slides [42–44]. An increased time to diagnose may be of particular concern for financially pressed health care systems. However, new image analysis programs, improvement of digital systems and increase pathologists' experience may decrease reading time in the future and rendering WSI a cost-saving tool, as suggested by a projected cost savings study that has calculated a $18 million saving by implementation of an enterprise-wide WSI system over a 5-year period [45].

Our study has several limitations. First, due to the retrospective design, we cannot exclude some bias. Second, the number of patients evaluated is limited to only 60. This number, however, represents the minimal number of patients recommended in a validation study based on the Guidelines previously mentioned [40]. Moreover, very heterogeneous diagnoses were chosen for the study, encompassing almost all pancreatic pathologies causing a pancreatic mass. Third, we did not use professional monitors such as those usually recommended. Our results, however, showed a very good concordance between LM and WSI despite this limitation. Fourth, the evaluation has been performed by expert pathologists, from high volume centers for pancreatic diseases, thus similar outcomes might not be replicated outside of this setting. Finally, we performed evaluation only of H&E stained samples without including specimens after immunostaining, which can be one of the major point of strength of WSI [7,8].

In conclusion, our results show a high concordance between light microscopy and whole slide imaging, as well as, a substantial inter-observer agreement and a complete intra-observer agreement regarding diagnostic classification on EUS-guided cell-block or histological acquired biopsy samples from patients with pancreatic solid lesions. Methods to decrease WSI reading time and make it more cost-effective to use digital images will be required for wider adoption of this technique in clinical practice.

Conflict of interest

None of the authors has any conflict of interest with the present manuscript.

Funding sources

The work had no specific funding.

Financial disclosures

Authors have nothing to disclose.

References

[1] Weinstein RS, Graham AR, Lian F, Braunhut BL, Barker GR, Krupinski EA, et al. Reconciliation of diverse telepathology system designs. Historic issues and implications for emerging markets and new applications. APMIS 2012;120(4):256–75.
[2] Al-Janabi S, Huisman A, Van Diest PJ. Digital pathology: current status and future perspectives. Histopathology 2012;61(1):1–9.
[3] Gilbertson J, Yagi Y. Histology, imaging and new diagnostic work-flows in pathology. Diagn Pathol 2008;3(Suppl 1):S14.
[4] Aeffner F, Blanchard TW, Keel MK, Williams BH. Whole-slide imaging: the future is here. Vet Pathol 2018;55(4):488–9.
[5] Farahani N, Pantanowitz L. Overview of Telepathology. Clin Lab Med 2016;36(1):101–12.
[6] 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):542–50.
[7] Hardy LB, Fitzgibbons PL, Goldsmith JD, Eisen RN, Beasley MB, Souers RJ, et al. Immunohistochemistry validation procedures and practices: a College of American Pathologists survey of 727 laboratories. Arch Pathol Lab Med 2013;137(1):19–25.
[8] Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human epidermal growth factor receptor 2 testing in breast Cancer: American society of clinical oncology/college of American pathologists clinical practice guideline focused update. Arch Pathol Lab Med 2018;142(11):1364–82.
[9] Bejnordi BE, Zuidhof G, Balkenhol M, Hermsen M, Bult P, van Ginneken B, et al. Context-aware stacked convolutional neural networks for classification of breast carcinomas in whole-slide histopathology images. J Med Imaging (Bellingham) 2017;4(4):044504.
[10] Maeda I, Kubota M, Ohta J, Shinno K, Tajima S, Ariizumi Y, et al. Effectiveness of computer-aided diagnosis (CADx) of breast pathology using immunohistochemistry results of core needle biopsy samples for synaptophysin, oestrogen receptor and CK14/p63 for classification of epithelial proliferative lesions of the breast. J Clin Pathol 2017;70(12):1057–62.
[11] Aeffner F, Martin NT, Peljto M, Black JC, Major JK, Jangani M, et al. Quantitative assessment of pancreatic cancer precursor lesions in IHC-stained tissue with a tissue image analysis platform. Lab Invest 2016;96(12):1327–36.
[12] Yoshida H, Yamashita Y, Shimazu T, Cosatto E, Kiyuna T, Taniguchi H, et al. Automated histological classification of whole slide images of colorectal biopsy specimens. Oncotarget 2017;8(53):90719–29.
[13] Lam AK, Leung M. Whole-slide imaging for esophageal adenocarcinoma. Methods Mol Biol 2018;1756:135–42.
[14] Camparo P, Egevad L, Algaba F, Berney DM, Boccon-Gibod L, Comperat E, et al. Utility of whole slide imaging and virtual microscopy in prostate pathology. APMIS 2012;120(4):298–304.
[15] Leo P, Lee G, Shih NN, Elliott R, Feldman MD, Madabhushi A. Evaluating stability of histomorphometric features across scanner and staining variations: prostate cancer diagnosis from whole slide images. J Med Imaging (Bellingham) 2016;3(4):047502.
[16] Kinoshita Y, Watanabe K, Ishii H, Kushima H, Fujita M, Nabeshima K. Proliferation of elastic fibres in idiopathic pulmonary fibrosis: a whole-slide image analysis and comparison with pleuroparenchymal fibroelastosis. Histopathology 2017;71(6):934–42.
[17] Shah KK, Lehman JS, Gibson LE, Lohse CM, Comfere NI, Wieland CN. Validation of diagnostic accuracy with whole-slide imaging compared with glass slide review in dermatopathology. J Am Acad Dermatol 2016;75(6):1229–37.
[18] Gilbertson JR, Ho J, Anthony L, Jukic DM, Yagi Y, Parwani AV. Primary histologic diagnosis using automated whole slide imaging: a validation study. BMC Clin Pathol 2006;6:4.
[19] Li X, Liu J, Xu H, Gong E, McNutt MA, Li F, et al. A feasibility study of virtual slides in surgical pathology in China. Hum Pathol 2007;38(12):1842–8.
[20] Mukhopadhyay S, Feldman 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.
[21] Vergani A, Regis B, Jocolle G, Patetta R, Rossi G. Noninferiority diagnostic value, but also economic and turnaround time advantages from digital pathology. Am J Surg Pathol 2018;42(6):841–2.
[22] Boyce BF. An update on the validation of whole slide imaging systems following FDA approval of a system for a routine pathology diagnostic service in the United States. Biotech Histochem 2017;92(6):381–9.
[23] Evans AJ, Bauer TW, Bui MM, Cornish TC, Duncan H, Glassy EF, et al. US food and drug administration approval of whole slide imaging for primary diagnosis: a key milestone is reached and new questions are raised. Arch Pathol Lab Med 2018;142(11):1383–7.
[24] Dumonceau JM, Deprez PH, Jenssen C, Iglesias-Garcia J, Larghi A, Vanbiervliet G, et al. Indications, results, and clinical impact of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline — Updated January 2017. Endoscopy 2017;49(7):695–714.
[25] Erickson RA, Sayage-Rabie L, Beissner RS. Factors predicting the number of EUS-guided fine-needle passes for diagnosis of pancreatic malignancies. Gastrointest Endosc 2000;51(2):184–90.
[26] Jhala NC, Jhala DN, Chhieng DC, Eloubeidi MA, Eltoum IA. Endoscopic ultrasound-guided fine-needle aspiration. A cytopathologist's perspective. Am J Clin Pathol 2003;120(3):351–67.
[27] Khurana KK, Graber B, Wang D, Roy A. Telecytopathology for on-site adequacy evaluation decreases the nondiagnostic rate in endoscopic ultrasound-guided fine-needle aspiration of pancreatic lesions. Telemed J E Health 2014;20(9):822–7.
[28] Marotti JD, Johncox V, Ng D, Gonzalez JL, Padmanabhan V. Implementation of telecytology for immediate assessment of endoscopic ultrasound-guided fine-needle aspirations compared to conventional on-site evaluation: analysis of 240 consecutive cases. Acta Cytol 2012;56(5):548–53.
[29] Bang JY, Hebert-Magee S, Navaneethan U, Hasan MK, Hawes R, Varadarajulu S. Randomized trial comparing the Franseen and Fork-tip needles for EUS-guided

fine-needle biopsy sampling of solid pancreatic mass lesions. Gastrointest Endosc 2018;87(6):1432–8.
[30] Rimbas M, Crino SF, Gasbarrini A, Costamagna G, Scarpa A, Larghi A. EUS-guided fine-needle tissue acquisition for solid pancreatic lesions: finally moving from fine-needle aspiration to fine-needle biopsy? Endosc Ultrasound 2018;7(3):137–40.
[31] Pitman Bishop M, Layfield L. The papanicolau society of cytopathology system for reporting pancreatobiliary cytology. Springer; 2015.
[32] Mitri RD, Rimbas M, Attili F, Fabbri C, Carrara S, Di Maurizio L, et al. Performance of a new needle for endoscopic ultrasound-guided fine-needle biopsy in patients with pancreatic solid lesions: a retrospective multicenter study. Endosc Ultrasound 2018;7(5):329–34.
[33] Mroz P, Parwani AV, Kulesza P. Central pathology review for phase III clinical trials: the enabling effect of virtual microscopy. Arch Pathol Lab Med 2013;137(4):492–5.
[34] Hipp JD, Lucas DR, Emmert-Buck MR, Compton CC, Balis UJ. Digital slide repositories for publications: lessons learned from the microarray community. Am J Surg Pathol 2011;35(6):783–6.
[35] Goacher E, Randell R, Williams B, Treanor D. The diagnostic concordance of whole slide imaging and light microscopy: a systematic review. Arch Pathol Lab Med 2017;141(1):151–61.
[36] 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.
[37] Evered A, Dudding N. Accuracy and perceptions of virtual microscopy compared with glass slide microscopy in cervical cytology. Cytopathology 2011;22(2):82–7.
[38] Bang JY, Kirtane S, Krall K, Navaneethan U, Hasan M, Hawes R, et al. In memoriam: fine-needle aspiration, birth: fine-needle biopsy: the changing trend in endoscopic ultrasound-guided tissue acquisition. Dig Endosc 2019;31(2):197–202.
[39] Shugang X, Hongfa Y, Jianpeng L, Xu Z, Jingqi F, Xiangxiang L, et al. Prognostic value of SMAD4 in pancreatic cancer: a meta-analysis. Transl Oncol 2016;9(1):1–7.
[40] 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):1710–22.
[41] Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016;531(7592):47–52.
[42] Gui D, Cortina G, Naini B, Hart S, Gerney G, Dawson D, et al. Diagnosis of dysplasia in upper gastro-intestinal tract biopsies through digital microscopy. J Pathol Inform 2012;3:27.
[43] Jen KY, Olson JL, Brodsky S, Zhou XJ, Nadasdy T, Laszik ZG. Reliability of whole slide images as a diagnostic modality for renal allograft biopsies. Hum Pathol 2013;44(5):888–94.
[44] Velez N, Jukic D, Ho J. Evaluation of 2 whole-slide imaging applications in dermatopathology. Hum Pathol 2008;39(9):1341–9.
[45] Ho J, Ahlers SM, Stratman C, Aridor O, Pantanowitz L, Fine JL, et al. Can digital pathology result in cost savings? A financial projection for digital pathology implementation at a large integrated health care organization. J Pathol Inform 2014;5(1):33.