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REVIEW ARTICLE

Artificial intelligence in cytopathology: a review of the literature and overview of commercial landscape

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Artificial intelligence (AI) has made impressive strides recently in interpreting complex images, thanks to improvements in deep learning techniques and increasing computational power. Researchers have started applying these advanced techniques to pathology images, although most efforts have been focused on histopathology. Cytopathology, however, remains the original field of pathology for which AI models for clinical use were successfully commercialized, to assist with automating Papanicolaou test screening. Recent AI efforts have focused on whole slide images of both gynecologic and non-gynecologic cytopathology. This review summarizes the literature and commercial landscape of AI as applied to cytopathology.
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Introduction: what is artificial intelligence?

Artificial intelligence (AI) is the branch of computer science concerned with development of machines with the ability "to perform those activities that are normally thought to require intelligence."[1] It is a broad field that encompasses several sub-categories such as machine learning, robotics, and knowledge representation, among others. The scope of this review will be on machine learning, particularly as it has been applied to cytopathology. In machine learning, the goal is to develop algorithms that learn from data and make predictions without being explicitly programmed how to do so. In supervised machine learning, an algorithm is supplied with a "training" data set that includes input data and its associated output (or "label") data. It then builds a mathematical model that fits the input data to the output data. Generally, the model is then supplied with a "validation" data set, on which the model based on the training data set is evaluated and tuned. Finally, the model is supplied with a "test" data set, and the fitness of the final model is evaluated. In unsupervised machine learning, labeled data is not provided, and the goal of the algorithm is to identify patterns within the data. For this review, *machine learning* will refer to supervised approaches.

Until recently, machine learning attempted to "automate the reasoning processing of experts" based on hand-crafted features.[2] For instance, an algorithmic approach to discriminate squamous cells might be based on features like cell shape, cytoplasm color, nuclei, and perhaps tens or hundreds of other defined features. A machine learning algorithm would create a multivariate model that incorporates any number of the supplied features to identify an abnormal squamous cell. In other words, the raw data set is first manipulated into a set of features that are understandable by a human before being presented to the algorithm. Over the last decade or so, deep learning has made significant advances and in many cases outperformed traditional computer vision models based on human-extracted features. *Deep learning* refers to computational techniques that extract hierarchies of features without the need for a human to define features to extract (Fig. 1). In a deep learning approach, the algorithm requires only a labeled training set (for instance, the pixel data of the squamous cell pictures with their corresponding diagnosis labels) from which it will "learn" the features for the model by itself. A tradeoff with deep learning is that the learned features typically would not correspond to human-understandable features. Instead, they are typically uninterpretable because they depend on complex interactions with other uninterpreted features.[2] Thus, although a deep learning algorithm may be more accurate, it is essentially a "black box"—meaning that how it arrives at an answer for a given case remains essentially unknown, and this could differ each time the algorithm is run as the model learns with each iteration. Another tradeoff with the deep learning approach is that these algorithms typically require much larger labeled data sets to develop compared with approaches based on hand-crafted features. Techniques have been recently developed, however, that allow for a deep learning model to be developed with a smaller data set. In a technique called "transfer learning," a model developed for one set of images can be transferred to another unrelated set of images and essentially re-tuned with the new data.

The most common deep learning approach is the deep neural network (DNN), a set of algorithms designed to recognize patterns and modeled after the human brain.[2] It is composed of layers of artificial "neurons" between the input

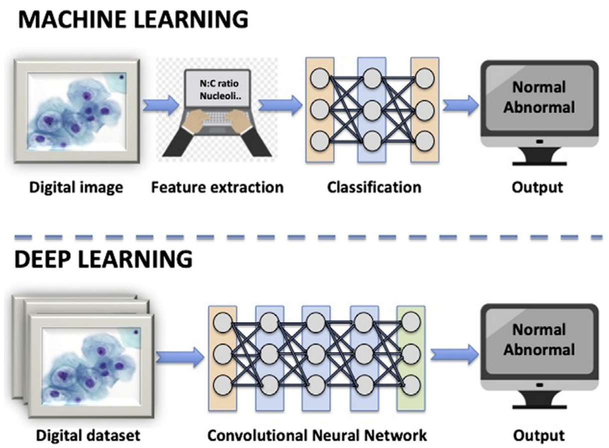

Figure 1 Different AI models. Top, Machine learning involves algorithms that parse data, use hand-crafted features to statistically learn from that data, and then apply what was learned to make a prediction or decision. Bottom, Deep learning requires large amounts of data and uses a layered convolutional neural network with an input, many hidden, and an output layer that can program itself to make intelligent decisions on its own.

Table 1 Applications of artificial intelligence to non-gynecologic cytology.

Specimen type	Image analysis details	Algorithm performance	Authors
Thyroid fine-needle aspiration			
	Feature-based k-nearest neighbor classifier that used 4 metrics based on nuclear roundness and chromatin texture.	Distinguished benign from malignant thyroid lesions with success rate of 75%.	Cochand-Priollet et al. 2006
	Total nuclear area and cytoplasmic area of cell clusters were calculated using a color deconvolution algorithm.	N/C ratio of 0.5 or less had odds ratio for malignancy of 4.6. Cases with N/C ratio of 0.5 or less and greater than 20 cell groups had an odds ratio for malignancy of 7.0.	Collins and Collins 2013
	Feature-based neural network (learning vector quantization) that used metrics based on nuclear size, shape, and texture.	Distinguished benign from malignant thyroid follicular cells with sensitivity of 91.5% and specificity of 92.4%.	Varlatzidou et al. 2011
	Feature-based neural network based on features scored by a human cytopathologist (eg, nuclear pseudoinclusions, pseudopapillary pattern, and cell monolayers) and clinical features (eg, age, sex, and size).	Sensitivity of 0.86 and specificity of 0.59 for detecting PTC.	Ippolito et al. 2004
	A convolutional neural network (ie, not based on defined features) was trained to distinguish papillary thyroid carcinoma from other thyroid lesions such as colloid nodules, follicular neoplasms, and lymphocytic thyroiditis.	Distinguished papillary thyroid carcinoma from other thyroid lesions with a sensitivity of 90.5%, specificity of 83.3%, negative predictive value of 96.5%, and diagnostic accuracy of 85.1%.	Sanyal et al. 2018
	Text-based support vector matrix that used 33 categories of synonymous keywords/phrases derived from microscopic descriptions of thyroid FNAs.	Distinguished classical PTC from NIFTP (as diagnosed on resection) in 81.1% of cases. Sensitivity of 88.9% and specificity of 96.0% in detecting classical PTC.	Maleki et al. 2018
Urine			
	Feature-based neural network (back propagation) using 25 morphometric features was trained on voided urine smears with histologic and/or clinical follow-up.	Detected urothelial carcinoma with sensitivity of 94.5% and specificity of 100%.	Pantazopoulos et al. 1998
	Feature-based neural network (back propagation) using 5 morphometric features with histologic and/or clinical follow-up.	Distinguished benign from urothelial carcinoma in all cases, and also distinguished almost all high-grade urothelial carcinoma from low-grade urothelial carcinoma.	Muralidaran et al. 2015
	A method combining N/C ratio measurement with deep-learning for assessment of nuclear atypia using whole slide images that rearranges the WSI to a grid format, and generates statistics for every cell, and outputs a suggested diagnostic category.	Not assessed yet.	Vaickus et al. 2019

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Table 1 (*continued*)

Specimen type	Image analysis details	Algorithm performance	Authors
Pancreatobiliary			
	Feature-based neural network that used metrics based on nuclear size, shape, and chromasia.	Distinguished benign from malignant cell clusters in pancreatic FNA with 83.9% accuracy.	Momeni-Boroujeni et al. 2017
	Deep neural network (single shot multibox detector).	Distinguished benign from malignant pancreatic FNA with 80% sensitivity and specificity of 80%.	Hashimoto et al. 2018
	Group area, individual nuclear area, number of nuclei per group, N/C ratio, and nuclear size differential were calculated.	An average nuclear area >70 μm^2 had a 3.2 risk ratio for suspicious categorization.	Collins and Weimholt 2015
Lung			
	Convolutional neural network trained on exfoliative and fine needle aspirate specimens from lung cancers into adenocarcinoma, squamous cell carcinoma, and small cell carcinoma. Assessed model with 3-fold cross validation.	71.1% accuracy.	Teramoto et al. 2017
	Described a method to identify *Mycobacterium tuberculosis* in Ziehl-Neelsen stained slides using color segmentation and shape extraction.	Authors report that the algorithm successfully detects most single bacilli, while overlapping bacilli are labeled as "possible."	Sadaphal et al. 2008
	Described a feature-based image ranking algorithm that presents first high-power fields with highest likelihood of containing acid fast bacilli.	Author reports that the algorithm successfully ranked acid fast bacilli-containing images as the highest in the data sets, despite only single bacilli being present in sparse images and despite tissue and staining artifacts.	Tadrous 2010
Breast fine-needle aspiration			
	Receiver operator curve analysis on nuclear morphometry parameters to determine optimum cutoffs for distinguishing benign and malignant breast FNA cases.	Nuclear size parameters distinguished between benign and malignant breast lesions with up to 81.2% sensitivity and up to 100% specificity in training set and correctly classified 7 out of 8 atypical ductal hyperplasia specimens as benign or malignant based on final histopathology diagnosis.	Kashyap et al. 2017
	Feature-based neural network (back-propagation) using morphometric features and qualitative features scored by a human cytopathologist.	Successfully classified all cases of benign and invasive lobular carcinoma cases and 6 of 7 invasive ductal carcinoma cases.	Dey et al. 2013
	Feature-based neural network (backpropagation algorithm) with some features automatically quantified (eg, nuclear size, density, and texture metrics) and others scored by a human cytopathologist (eg, cellularity, prominence of nucleoli, and mitotic rate).	Sensitivity and specificity of 100% in distinguishing fibroadenomas from infiltrating carcinomas in test set.	Subbaiah et al. 2014

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Table 1 (*continued*)

Specimen type	Image analysis details	Algorithm performance	Authors
	Identified nuclei using circular Hough transform and filtered them for high-quality estimations. Developed 4 different feature-based models (k-nearest neighbor, naïve Bayes classifier, decision tree, and support vector machine) using 25 parameters for distinguishing benign and malignant breast lesions.	Sensitivity at the patient level of up to 0.88 and specificity of up to 1.0.	Filipczuk et al. 2013
Pleural effusions			
	Feature-based model (k-nearest neighbor) based on chromatin distribution metrics.	Distinguished malignant mesothelioma from benign mesothelial cells with 100% sensitivity and specificity.	Tosun et al. 2015
Endometrium			
	Feature-based model (classification and regression trees) for distinguishing benign and malignant endometrial lesions.	Distinguished malignant from benign cases with a sensitivity of 72.1% and specificity of 90.7%.	Pouliakis et al. 2014

Abbreviations: FNA, fine-needle aspiration; N/C ratio, nuclear-to-cytoplasmic ratio; PTC, papillary thyroid carcinoma; WSI, whole-slide imaging.

and output layer. Each layer includes one or more neurons linked with neurons in the previous and next layers, and the weights of the connections are adjusted until the model best fits the data.[3] A convolutional neural network (CNN) is a neural network that is used specifically for complex image interpretation. Its name comes from the fact that it contains one or multiple "convolutional" layers, which measure each feature at every position in the image. This allows features in subsequent images to be identified regardless of their location within the image. Neural networks have become much more effective tools in the last decade because increases in computational power have enabled them to incorporate more layers and thus more hierarchical complexity.

AI in health care

The literature dealing with computer-based clinical decision support in health care dates back to almost 60 years ago.[4] Following the widespread adoption of interconnected electronic health records and digital imaging, AI has made impressive contributions to health care, which have begun to garner headlines in mainstream media.[5] Given the strengths of deep learning in intricate pattern recognition and predictive model building from big data, this technology is very appealing for health-related applications. Several innovative software solutions and medical devices have demonstrated diagnostic accuracy that matches the performance of clinical experts.[6-8] For instance, a Nature study published by Esteva et al[6] in 2017 described a deep CNN based on photographic and dermoscopic images of skin lesions that distinguished keratinocyte carcinomas from benign seborrheic keratoses and malignant melanomas from benign nevi with an accuracy comparable to dermatologists. They suggested that their model could be deployed in the clinic as a mobile app by dermatologists and even primary care providers to help decide whether a skin lesion is appropriate to biopsy. The subject of AI in health care became particularly interesting in recent years when Google developed a deep learning algorithm to detect diabetic retinopathy in retinal fundus photographs that was on par with experts.[9] Moreover, in 2018 the US Food and Drug Administration (FDA) approved an AI stroke application[10] signaling a positive shift in the regulatory process for computer-aided diagnostic software. In order to be successful, an AI-based clinical decision support tool needs to be relevant (ie, solve an actual problem), efficient (ie, save time and not disrupt workflow), scientifically sound (eg, peer-reviewed scientific evidence establishing validity and safety), simple to use, and easily understood by users.[4]

AI in anatomic pathology

Researchers have attempted to use machine learning for decades to accurately interpret pathology images. In recent years, the increased use of whole-slide imaging (WSI) coupled with advances in computation and deep learning methods has sparked much interest in applying AI to pathology.[11,12] This was spurred by recent high-profile competitions hosted by international societies. For instance, the International Conference for Pattern Recognition in 2012 hosted a competition for mitosis detection in histology images of breast cancer based on a publicly available annotated data set. The winning group[13] used a deep CNN approach that outperformed other competing techniques by a significant margin. The International Symposium on Biomedical Imaging has since hosted the CAMELYON (Cancer Metastases in Lymph Nodes) challenge. The first challenge, which ended in 2016, was to evaluate new and existing algorithms for the automated detection of metastases in hematoxylin and eosin—stained whole slide images of lymph node sections. In fact, this was the first challenge ever to use whole slide images, with a data set that was over 600 GB.[14] The training data set included whole slide images from 2 centers in the Netherlands with and without nodal metastases verified by immunohistochemical staining. The top 5 algorithms in the competition, as reported in the *Journal of the American Medical Association*, had a mean area under the curve comparable to the study pathologist interpreting the slides without time constraints.[15] The next CAMELYON 2017 challenge, which is still underway, combines the assessment of multiple lymph node slides into 1 pN-stage. Once again, the fact that Google participated in this deep learning challenge[16] has roused much awareness.

Several other studies have used machine learning to not only detect and grade cancer, but to also predict the prognosis of patients with cancer based on pixels in pathology digital images.[17] For instance, a 2016 study developed a feature-based model for accurately predicting the prognosis of patients with stage 1 non-small cell lung carcinoma using whole slide images.[18] Interestingly, the authors provided examples of low-grade tumors for which the model correctly predicted shorter survival and high-grade tumors for which the model correctly predicted longer survival. Computational pathology studies have also demonstrated that deep learning diagnoses can facilitate clinical decision making by identifying cases at high risk of misdiagnosis,[19] or even assist pathologists with predicting the most commonly mutated genes in certain cancers.[20] In recent years there has also been much interest in applying deep learning methods to study the tumor microenvironment. Interestingly, several studies using deep CNNs have been published showing that the evaluation of tumor stroma alone is very useful for classifying malignant lesions and determining their prognosis.[21,22]

Several companies have developed or are actively developing proprietary machine learning algorithms that automate repetitive and time-consuming tasks for pathologists, grade aggressiveness of tumors, and discover predictive biomarkers.[23] Some commercial companies devoted to developing AI tools for anatomical pathology include Lunit, Ibex, aetherAI, DeepBio, PathAI, Paige.AI, Nucleai, MechanoMind, ContextVision, Fimmic, Visiopharm, Huron, Proscia, and Indica Labs, among others. To develop their algorithms, many app developers and research groups have accessed public whole slide image data sets (eg, The Cancer Genome Atlas [TCGA])[24] and/or started to form alliances with pathology laboratories. Pairing genomics and clinical metadata with whole slide images provided by TCGA has offered investigators a unique opportunity to perform correlative studies.[25] Now that WSI has been approved in the US by the FDA for primary diagnosis in surgical pathology and progressively more pathology laboratories have started purchasing such WSI systems, these algorithms are expected to significantly impact the practice of pathology. Also, this may be facilitated by the fact that the FDA is in the process of developing a program that will provide more streamlined regulatory oversight of software-based medical devices, thereby enabling faster commercialization of these algorithms.[26]

AI in cytology

Lessons learned from automating the Papanicolaou smear

Although most current efforts at applying machine learning to pathology have been focused mainly on histopathology, perhaps 1 of the earliest and most commercially successful AI algorithms in anatomic pathology targeted cytopathology. Automating screening of the Papanicolaou smear was an obvious initial target for the application of machine learning given the tedious and repetitive nature of the task and the sheer volume of the test, which enabled economy of scale. One of the first commercially available automation-assisted systems designed for this purpose was PAPNET. PAPNET was developed by Neuromedical Systems Inc., (NSI) and was introduced around 1992. It was designed to detect cervical epithelial abnormalities missed on prior manual microscopic examination of conventional Papanicolaou smears.[27] Glass slides had to be mailed to a central scanning station, which scanned and analyzed them, with the system utilizing a hierarchical design incorporating both algorithmic image and neural network processing. The algorithmic image processing was designed to identify objects including cells and cell clusters, using features such as gray-scale intensity, size, and local contrast. The neural network received the object images and gave each one a numerical score based on its resemblance to the abnormal cells in the training set. Color images of 128 potentially abnormal events were copied onto digital tape, which was then returned with the slides to the participating cytology laboratory. Once returned, a cytotechnologist would review these 128 images. If any images appeared abnormal, the cytotechnologist would re-examine the slide at their light microscope. If all the images appeared normal to the cytotechnologist, however, then no further examination would be done. Because of the reluctance of most laboratories to ship slides to a national center and wait to have them returned, NSI developed PAPNET-on-Cyte, which, beginning in 1997, was available for installation at the customer's laboratory and allowed for both screening and diagnosis to be performed on premises.[28] PAPNET struggled to gain market acceptance, however, and eventually NSI filed for bankruptcy in 1999;[29] their intellectual property, patents, and assets were purchased by AutoCyte (later called BD Tripath).

One of the main lessons learned from the PAPNET experience was that rescreening negative cases for only modest improvements in sensitivity was not a strong-enough value proposition to entice laboratories to purchase an expensive system that also delayed their turnaround time to screen cases. Subsequent attempts at applying machine learning to cervical cytology instead aimed to assist or replace primary screening. The ThinPrep Imaging System (Hologic, Marloborough, MA) and FocalPoint GS Imaging System used with SurePath slides (Becton Dickinson, Franklin Lakes, NJ) filled this void and today continue to dominate the market. The ThinPrep Imager, which emerged in 2004, uses a proprietary algorithm based on several cellular features to identify the 22 most concerning fields of view (FOVs) on a ThinPrep slide. A cytotechnologist is subsequently directed to the coordinates of these FOVs using a robotic microscope. If any of the fields are deemed abnormal, then the entire slide must be screened manually; but if all 22 fields are considered to be normal then the slide can be signed out as negative with no need for further glass slide review. The FocalPoint GS Imaging System, which was originally called AutoPap, was FDA-approved for primary screening of conventional smears in 1998. It was designed to provide a risk category for entire slides. Those with a risk of abnormal cells of less than 25% could be signed out as negative without any human review. The AutoPap has since been approved for use with SurePath liquid-based cytology in 2001. It was not as commercially successful as the ThinPrep Imaging System, so it was also modified into a guided screening (GS) system with a robotic microscope, which received FDA approval in 2008 and was called the FocalPoint GS Imaging System. This latter system identifies 10 FOVs considered to represent the most abnormal material, which are to be reviewed by a cytotechnologist. Any abnormality in these FOVs, as determined by the cytotechnologist, requires manual screening of the entire slide. Thus, every slide undergoes at least partial human review. For a more detailed overview of automated Papanicolaou test screening, we refer the reader to an excellent recent review by Thrall.[30] A newer image analysis system for automated Papanicolaou test screening known as BestCyte from CellSolutions (Greensboro, NC) is in development. With this system, abnormal cells are grouped and displayed in gallery format. So far, at least 1 study has demonstrated that the BestPrep liquid-based thin-layer Papanicolaou test and BestCyte cell sorter imaging system is equivalent to ThinPrep for manual review.[31]

There are several take-home lessons from these early computational cytopathology efforts. Vendors that were successful owned the entire process including both pre-imaging factors (eg, sample fixation, standardized processing and staining, creating a monolayer) and imaging steps (eg, slide scanning and image analysis). Despite initial criticism, these systems were adopted by cytology laboratories into their routine clinical workflow because the solution addressed an actual problem (ie, the need for automation and improved accuracy) and there was additional reimbursement for screening using an automated system.[32] For instance, the current procedural terminology (CPT) code 88175 can be applied for a liquid-based Papanicolaou test with screening by an automated system. Almost 2 decades ago, the FDA also approved screening pathology slides without the need for human review. Most of these factors (eg, pre-imaging standardization, billing) have yet to be addressed in the field of surgical pathology. A recent review of the image analysis and machine learning techniques previously utilized for automated Papanicolaou test screening indicated that there were weaknesses in these techniques, resulting in low accuracy.[33] These authors also highlight the deficit of evidence that these algorithms will work in clinical settings found in developing countries. Fortunately, several researchers have continued to optimize the application of machine and deep learning to cervical cytology,[34-38] presumably with the eventual aim of precluding any requirement for human review. Zhang et al[39] applied CNNs for classifying cervical cytology images into benign and malignant. They used 2 publicly available annotated data sets and achieved accuracies of approximately 98%. Their model was unique in that it used no handcrafted features or segmentation between nucleus and cytoplasm. They have not yet reported the performance of their model on whole slide images. Martin et al[40] reported that they applied CNNs to whole slide images of ThinPrep slides prepared from cervical Papanicolaou smears. They annotated cells of interest and developed a model that attempted to distinguish the 5 different categories in The Bethesda System. They reported an overall average accuracy of 60%. Moreover, it appears that directing end users to review only a few images in a gallery of diagnostically relevant cells on a computer monitor may be more efficient than having them review an entire slide using a robotic microscope or whole slide image.[41]

Applications of AI to non-gynecologic cytology

Although most computational cytopathology efforts have been applied to working with Papanicolaou test images, there have been several published studies over the last 2 decades that have also applied machine learning to non-gynecologic cytology. Most of these studies used feature-based algorithms on representative human-selected photomicrographs. A few studies in the late 1990s and early 2000s tested the PAPNET system, initially designed for cervical cytology, with several other types of cytology specimens. Vriesema et al[42] applied the PAPNET system to distinguish benign, low-grade, and high-grade urothelial carcinoma on bladder washing specimens. The gold standard in their study was cystoscopy findings with or without histologic confirmation. They found that the diagnosis made with PAPNET had a higher area under the curve by receiver operating characteristic curve analysis (0.71) than light microscopy-based diagnosis (0.58). Similar studies were undertaken using the PAPNET system on sputum samples for the detection of lung carcinoma,[43] on esophageal cytologic specimens,[44] and on oral cytologic specimens for the detection of oral squamous cell carcinoma.[45] Applications of AI to non-gynecologic cytology are described below and are summarized in Table 1.

Thyroid gland aspirations

In 2005, Cochand-Priollet et al used 25 extracted nuclear features from thyroid FNA cases and found that 4 features based on nuclear shape, chromatin texture, and distribution of chromatin were important when discriminating benign from malignant thyroid lesions.[46] The malignant category included mostly cases of papillary carcinoma, as well as a few cases of Hürthle cell carcinoma, medullary carcinoma, and follicular carcinoma. Their model had a success rate of up to 75.25% in their test set. A pilot study using ImageJ to develop an image algorithm in order to analyze color photographs identified significant differences in multiple morphometric features (area, perimeter, and anisonucleosis).[47] However, these investigators noted that the average size of a papillary thyroid carcinoma nucleus shrunk to the size of a benign nucleus on direct smear. This highlights one of the machine learning challenges in cytology, due to multiple different slide preparations and stains. When Brian Collins evaluated whole slide images showing atypia of undetermined significance in thyroid fine-needle aspiration (FNA), he was able to establish defined objective criteria (eg, nuclear-to-cytoplasmic [N/C] ratio of 0.50 or less and greater than 20 cell groups) with the potential to provide an assessment of malignancy.[48] In a related study using whole slide images of a single air-dried Romanowsky-stained slide, Legesse et al showed that nuclear area and elongation were also predictive of the final diagnosis including noninvasive follicular thyroid neoplasm with papillary-like features (NIFTP).[49] Varlatzidou et al[50] performed a similar study in thyroid lesions in which they used extracted features to create a neural network model with a sensitivity in the test set of 91.5% and specificity of 92.43%. They specified that most nuclei selected for analysis were those of follicular and Hürthle cells, and they excluded stromal cells, histiocytes, and lymphocytes. The malignant group was composed mainly of papillary carcinoma, followed by medullary carcinoma and then anaplastic carcinoma. Their model had 2 components, with the first characterizing individual cell nuclei as benign or malignant and the second component characterizing the overall slide as benign or malignant based on the number of benign or malignant cells detected.

Ippolito et al[51] applied a neural network model to thyroid FNA cases with indeterminate cytology, using corresponding histologic diagnoses from resection specimens as a gold standard. Their model did not use digitally extracted features like the previous studies, but instead incorporated categorical features such as colloid, chronic inflammation, and nuclear atypia based on an expert cytologist's review. They also attempted to incorporate clinical features like maximum nodule diameter and presence of a multinodular goiter, although more specific ultrasound features were not incorporated. Perhaps, not surprisingly, the features that they found contributed to the overall performance of the model included nuclear pseudoinclusions and a pseudopapillary pattern, as well as "cell monolayers." Moderately contributing features included a microfollicular pattern, nuclear overlap, and nuclear grooves. They reported a sensitivity of 0.86 and specificity of 0.59 for their neural network, and a sensitivity of 0.29 and specificity of 0.82 for manual cytology. In a related study using photomicrographs of smears from papillary thyroid carcinoma and other thyroid lesions,[52] the artificial neural network that Sanyal et al developed showed good sensitivity (90.48%), moderate specificity (83.33%), and excellent negative predictive value (96.49%) as well as diagnostic accuracy (85.06%). However, one drawback these researchers encountered was vague papillary formations in benign cases being wrongly identified as papillary carcinoma.

Maleki et al[53] recently developed a text-based model based on microscopic descriptions of thyroid FNAs which were diagnosed on resections as either classical papillary thyroid carcinoma (cPTC) or NIFTP. They found that the model was successful in distinguishing cPTC from NIFTP in 81.1% of cases. It is not clear whether the cPTC category included follicular variant of papillary thyroid carcinoma (FVPTC) cases, as the distinction on cytology between NIFTP and FVPTC is notoriously more difficult than the distinction between NIFTP and classic PTC.

Urine specimens

The Paris System for Reporting Urinary Cytology includes quantitative criteria such as the number of atypical urothelial cells gs1:_501100009229 present (eg, <5-10 cells for suspicious for high-grade urothelial carcinoma) and N/C ratio (eg, 0.5 and 0.7 cutoff values).[54] However, such quantitation criteria are hard for humans to accurately reproduce visually for precise category assignment,[55] despite the fact that digital image analysis supports a N/C ratio cutoff value of 0.5 for atypical urothelial cells.[56] In a study evaluating urinary cytology specimens using the CellSolutions Best-Cyte Cell Sorter imaging system, researchers reported greater interobserver variability than routine manual review,[57] probably because this system was initially designed for analyzing cervical cytology cases.

Different groups of researchers have applied neural network models, based largely on nuclear morphometry features, to distinguish benign from malignant urothelial cells. Pantazopoulos et al[58] reported a sensitivity of 94.5% and specificity of 100% for detecting urothelial carcinoma in urine cytology specimens using a feature-based neural network. They used follow-up data including histologic examination, microbiological reports, clinical course, subsequent cytological examination, ultrasound, pyelography, and cystoscopy for their gold standard diagnosis. Muralidaran et al[59] developed a feature-based neural network model for distinguishing benign, low-grade urothelial carcinoma, and high-grade urothelial carcinoma but did not appear to include cystoscopic, histologic, or clinical follow-up for confirmation of the diagnosis, which makes their results difficult to interpret. Their overall algorithm performance numbers were less than those reported by Pantazopoulos et al. Sundling et al reported that they are refining a CNN approach for urine cytology[60] but have not yet published their results. Vaickus and Liu recently described a method that combines objective measurement of N/C ratio with deep learning for scoring of nuclear atypia utilizing whole slide images of urine cytology specimens.[61] The software segments the cells and cell clusters, which are displayed in a grid format and can be presented hierarchically based on analysis of their N/C ratio and/or atypia score.

Pancreaticobiliary specimens

Momeni-Boroujeni et al[62] developed a neural network model for pancreatic FNA based on nuclear morphometry features that distinguished benign from malignant cell clusters with 83.9% accuracy. They also tested their model on "atypical" cases in a novel way. Considering that "atypical" pancreatic FNA cases usually do not result in surgical resection, they used the time that a patient was alive and without a diagnosis of pancreatic cancer as a surrogate marker of malignancy at the time of the FNA. They found that those cases diagnosed as benign by the model had a significantly longer time to death or diagnosis of pancreatic cancer by Kaplan-Meier analysis (median time to diagnosis of cancer or death: 1174 days) compared with those diagnosed as malignant by the model (median time to diagnosis of cancer or death: 67 days). Hashimoto et al[63] used a deep learning technique on cytopathology images without handcrafted feature extraction. They applied a DNN to pancreatic FNA specimens that assigned them into "suggestive or suspicious of malignancy" or "suggestive of benign" categories. They reported a sensitivity of 80% and specificity of 80% and added that they anticipated they could improve the performance of the classifier by increasing the volume of the training set. In another study, Collins and Weimholt subjected bile duct brushing cases with an indeterminate diagnosis (atypical to suspicious) to WSI quantitative analysis.[64] They found that cases categorized as suspicious had more nuclear size pleomorphism and larger nuclei than those categorized as atypical. Further work, however, is required to utilize such objective criteria in an image algorithm to help support the diagnosis of cholangiocarcinoma.

Lung specimens

Teramoto et al[65] used a DNN to classify liquid-based cytology specimens from lung cancers into adenocarcinoma, squamous cell carcinoma, and small cell carcinoma with 71.1% accuracy. Their accuracy rate for distinguishing non-small cell carcinoma (adenocarcinoma or squamous cell carcinoma) from small cell carcinoma was 85.6%. However, the scope of their study was limited to distinguishing only subtypes of lung carcinoma and did not attempt to distinguish benign from malignant cases. Given the high demand for ancillary testing to be performed on non-small cell lung carcinoma (NSCLC), cytopathologists are frequently asked to determine FNA material adequacy prior to performing molecular testing. Not surprisingly, quantitative analysis of cellularity in digitized cell block material has been shown to be more reliable using software than visual estimation by cytopathologists.[66] Although a commercial machine learning algorithm (TissueMark, Philips, Amsterdam, the Netherlands) has been developed and validated for automating tumor analysis[67] (ie, for automated tumor annotation and percentage tumor nuclei measurement in NSCLC) in formalin-fixed paraffin embedded tissue sections, the use of similar AI tools has not yet been widely applied to cytology samples.

To date, there are only a few publications regarding the use of AI tools to analyze sputum samples. Several independent groups have published their findings using image processing methods for the detection of *Mycobacterium tuberculosis* from bright-field microscopic sputum images.[68-70] Although promising, many of these methods failed to accurately identify stained bacilli that were touching or overlapping (eg, forming a T-shaped structure), as these were classified as non-bacilli.[68] More recently, image algorithms have also been applied to three-dimensional (3D) images of abnormal epithelial cells detected in sputum that were acquired using the Cell-CT instrument (VisionGate, Phoenix, Ariz). The intent of this emerging technology is to use computers to not only process 3D cell features, but also automatically provide cell classifications,[71] similar to what was accomplished with the automated Papanicolaou test screening systems.

Breast samples

Nuclear morphometry studies on cytology breast specimens have shown that based on certain features (nuclear size, shape, texture, and density) it is possible to distinguish between benign and malignant breast lesions.[72] When Dey et al applied an artificial neural network to FNA smears of histology proven breast lesions, their algorithm was able to differentiate all of the benign and lobular carcinoma cases and the majority of ductal carcinoma cases.[73] Subbaiah et al[74] likewise developed a neural network model for distinguishing adenocarcinoma from fibroadenoma in breast FNAs based on a combination of several features quantified by nuclear morphometry and other features scored by a human cytopathologist. They reported a sensitivity in the test set of 100% and a specificity of 100%. Their model, however, was restricted to cases that were cytologically unequivocal and was not tested on difficult cases with histologic follow-up. Another drawback of their model was the fact that it required a cytopathologist to manually review and score specific features (such as cellularity, dissociation, bipolar cells, etc) as this itself would likely take significantly longer than conventional manual review. Filipczuk et al[75] developed several different models for distinguishing FNAs of benign and malignant breast lesions, for which they report a sensitivity at the patient level of up to 0.88 and specificity of up to 1.00.

Pleural effusions

To date, we are aware of only one study that applied machine learning methods to analyze images of body effusions. Tosun et al[76] developed a model based largely on chromatin distribution to distinguish malignant mesothelioma from benign mesothelial cells in 34 effusion specimens. They extracted morphological features (eg, area, convexity, circularity, perimeter, eccentricity, equivalent diameter), texture features (eg, Haralick and Gabor features), and wavelet-based features. Their analysis showed a sensitivity of 100% and specificity of 100%. All of the cases in that study had a concurrent or subsequent pleural biopsy, which served as the gold standard for the evaluation. Based on their findings, these authors believe that the nuclear structure of mesothelial cells alone may contain enough information to separate malignant mesothelioma from benign mesothelial proliferations.

Endometrium

Pouliakis et al[77] used classification trees based on nuclear morphometry features to distinguish benign from malignant endometrial liquid-based cytology cases. The specimens were taken by direct sampling of the endometrial cavity with the EndoGyn Sampler (Gima, Milano, Italy). They reported that their model had a sensitivity of 72.1% and specificity of 90.68% for malignancy, using histological examination of endometrial curettage and/or hysterectomy as the gold standard.

Conclusion

The use of machine learning and deep learning is slowly permeating the practice of cytology. We will likely continue to see a shift towards AI-based tools in our field thanks to better computation, cloud computing, and mass data compilation as more laboratories start using WSI scanners. Many lessons learned from the success achieved with automation-assisted Papanicolaou test screening can be applied to augmenting the diagnosis of non-gynecologic cytology specimens, as well as surgical pathology cases. This review article was based largely on available published literature. There are several contemporary academic and commercial efforts underway focusing on AI in cytology that have yet to be fully revealed. Numerous opportunities for utilizing AI in cytology practice exist that were not covered in this review, such as methods to automate and enhance rapid onsite evaluation (ROSE) and routine quality assurance tasks (eg, cytologic-histologic correlation, “5-year look back” retrospective rescreen).

Most of the studies reviewed used traditional machine learning methods that relied on hand-crafted features and human-selected photomicrographs or image tiles of representative cells. Hence, the application of AI to cytopathology lags behind recent developments seen with histopathology. In the future, more deep learning methods that interpret cytology specimens at the level of a whole slide image are expected. A unique challenge in cytology is the presence in many specimens of 3D clumps of cells, which require image acquisition and subsequent analysis in multiple focal planes. On the other hand, compared with surgical pathology slides, cytology specimens have less of a need for interpreting complex spatial relationships, aside from individual cell clusters. Many of the machine learning algorithms thus far have focused largely on cellular features of cytology specimens. Future efforts may attempt to also incorporate stromal elements and background material (eg, colloid, matrix, necrosis).

Having curated, high-quality data and an integrated digital pathology infrastructure in place is critical. As our cytology data sets become larger and computers become more powerful, the results achieved by deep learning will likely get better. Additional challenges anticipated to the widespread use of AI in cytology will be quantifying its financial value and overcoming the resistance of humans to using this technology. Undoubtedly, if developed and implemented well AI technology is anticipated to greatly augment cytology practice by enhancing operational processes such as automation and improving decision-making. Cytologists will need to not only adapt their practice, but also become more engaged in order to help better shape this technology so that it is suitable for application to our field.

Conflict of interest

Liron Pantanowitz consults for Ibex and Hamamatsu and is on the medical advisory board for Leica.

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