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Review Article

Digital pathology and artificial intelligence in veterinary medicine

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Abstract
This review discusses the benefits and limitations of using digital pathology and artificial intelligence in veterinary pathology. Digital pathology has the potential to build a global community of pathologists as it promotes communication and collaboration among professionals due to the ease of sharing scanned slides, allowing the flexibility of hybrid or remote work and reducing the need for travel. Artificial intelligence can assist pathologists in laborious tasks, such as counting mitoses and improving consistency in scoring systems. However, these technologies present limitations and risks that must be evaluated and managed carefully.
Keywords: algorithms; ethic; digitized slides.

Introduction

Anatomic pathology as a diagnostic tool relies on the pathologist's knowledge and precision in interpreting microscopic findings to accurately diagnose lesions in patients and guide therapeutic decisions. In this context, technologies such as digital pathology (DP) and artificial intelligence (AI) can assist pathologists in diagnosing and grading histological lesions, aiming to streamline processes to reach a more accurate and less labor-intensive final diagnosis. The COVID-19 pandemic has accelerated the implementation of DP, as lockdown measures and social distancing prompted many to work from home. Throughout this crisis, DP has enabled pathologists, residents, molecular biologists, and pathology assistants to remain engaged in the diagnostic process (16). The objective of this review is to provide an overview of the application of these technologies and discuss some ethical considerations surrounding their use.

Digital pathology

Digital pathology (also known as virtual microscopy) is the technology used to view histologic slides on an electronic screen (16, 18, 19). This is achieved by using scanning devices that provide high-resolution digital microscopic images that can be viewed on electronic devices (3, 16, 18, 20, 21). The advent of whole slide digitalization creates new opportunities for pathologists, including digital collaboration, teaching, and telepathology, allowing cases to be discussed in real time by experts from anywhere in the world (16, 17, 18). Furthermore, it is possible to store and integrate digitized slides with health records and to support diagnostics using computational tools such as AI (17).

To create a whole slide digital image, two main components are required: hardware and software (15). The hardware consists of a scanner or microscope with objectives, light sources (brightfield or fluorescent), robotics for loading and moving glass slides, high-resolution digital cameras for image capture, and a computer (13, 15, 19). The software includes computational programs capable of handling, managing, and visualizing digital slides. The process begins with sample collection and standard histologic preparation involving fixation, sectioning, embedding in paraffin, microtomy, staining, and drying (13). Subsequently, the slide scanning process starts, during which scanners capture numerous images of tissue sections side by side or in a line-scanning format (15).

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These multiple images are digitally stitched together by the software to generate a digital image of the entire slide. This image is then examined by the pathologist to obtain the histopathological diagnosis of the tissue (Figure 1) with a magnification and resolution comparable to a microscope. The standard format of the image files varies between different vendors, therefore the use of a universal image format such as DICOM (4) is preferred to allow for compatibility between software. Staining techniques for digitized slides can be categorized as brightfield, fluorescent, and multispectral. Some scanners can accommodate multiple modalities, allowing for both brightfield and fluorescent scanning (15, 13, 21). Brightfield scanning simulates standard brightfield microscopy, being the most routinely used and economical approach. Fluorescent scanning is used for scanning slides labeled with fluorochromes (e.g., immunofluorescence and fluorescent in situ hybridization), allowing for further examination of fluorescent signals and storage of images without fading (21).

Digitized slides are rich in information, such as color, tissue morphology, cellular morphology, and complex cellular phenotypes (3, 6, 8, 18). The evaluation of these complex tissues and cellular phenotypes requires specific knowledge acquired through years of training and experience in case studies and peer review sessions. However, visual assessment by pathologists can be influenced by cognitive and visual biases/tendencies. To overcome these challenges, several technologies utilize AI algorithms that assist pathologists in the recognition of subtle lesion patterns and aid in the diagnosis or new discoveries about disease pathogenesis (21).

DP is already widely adopted in various countries in Europe, the United States, Canada, and Australia, as a routine part of the diagnostic process in both human and veterinary medicine (13, 14). In Brazil, however, the use of DP is still at an early stage due to the high cost of equipment and the necessary infrastructure for its implementation and operation.

Artificial Intelligence

Artificial intelligence (AI) is a broad term used since the 1950s. AI refers to the branch of computer science in which approaches based on systems or machines are used to make predictions by simulating what an intelligent human can do in the same situation. Machine learning approaches in DP are

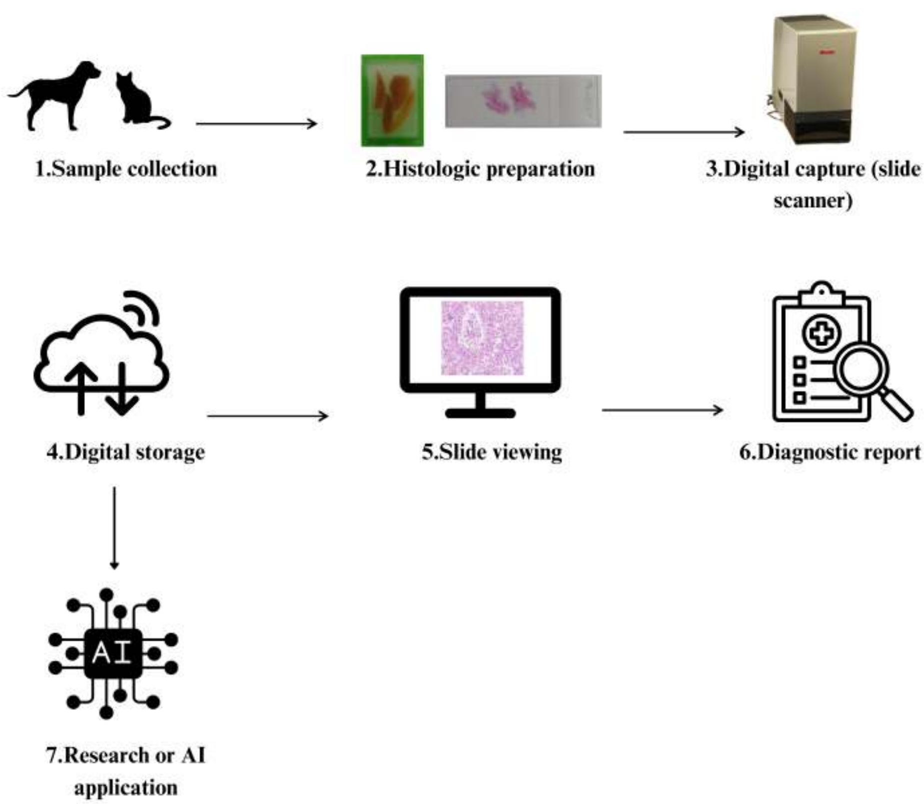

Figure 1. Workflow using digital pathology: the process begins with sample collection (1) and preparation (2) by fixation, cleavage, paraffin embedding, microtomy, staining, mounting and drying. After that, high-resolution scanning is performed using scanners (3). The image is then uploaded to a server (4) to be viewed by the pathologist at his workstation (5) to prepare a diagnostic report (6). The pathology report data is stored on the server along with the patient's image data as identifiable information. It is then de-identified upon export for research purposes or for building databases for Artificial Intelligence (7). Figure adapted from Ancheta et al. (2) and McKay et al. (14).

built on large databases and algorithms that identify patterns of lesion similarity and make predictions based on these (11).

In DP, AI can be applied to various tasks such as pattern recognition of lesions and predicting disease diagnoses, prognoses, and treatment responses based on image patterns. Several AI applications in DP focus on automating time-consuming tasks for pathologists, allowing them more time for high-level decision making, especially in diseases with ambiguous characteristics (5). A good example of AI application is the mitotic count in neoplasms. Mitotic figure counting is the oldest and widely used method to estimate cellular proliferation in tumors in a cost-effective manner. However, it is subjective, labor-intensive, and shows considerable variation among observers. Major causes of inconsistency in mitotic counts include variations in identifying mitotic figures and pathologist's fatigue from repetitive tasks (7). Standardized and validated AI algorithms capable of identifying mitotic figures can enhance diagnostic accuracy and relieve pathologists from the laborious task of counting.

AI also assists pathologists in the quantification of immunohistochemistry biomarkers associated with disease processes to determine prognosis and select treatments with higher efficacy (18). One example is the cell proliferation marker Ki-67. This marker is widely used across various types of neoplasms; however, there is intra- and inter-observer variability in determining the index, and manual quantification is time consuming. These challenges have prompted the search for more precise solutions in virtual microscopy aided by AI (10). For manual Ki-67 assessment, a conventional microscope and glass slide are used, requiring, in the case of canine oral melanomas, the counting of all immunolabeled cell nuclei in five fields at 400x magnification using a graticule (2). This counting is performed in areas with the highest nuclear labeling. The count and subsequent calculation generate the cell proliferation index of the tumor (2). The advantages of using DP combined with AI include improved standardization and reliability in the Ki-67 assessment, as well as enhanced speed and practicality for quantitative analyses compared to the current gold standard of manual evaluation (12).

Another example of AI utilization is computer-aided diagnosis demonstrated in a recent study by Fragoso-Garcia et al. (2023), in which an algorithm developed from training an artificial neural network was capable of automatically distinguishing and classifying seven major canine skin tumors: trichoblastoma, squamous cell carcinoma, peripheral nerve sheath tumor, melanoma, histiocytoma, mast cell tumor, and plasmacytoma. However, compared to the consensus of six pathologists who examined the tumors, the algorithm's diagnostic performance was slightly inferior. The use of algorithms for the diagnosis in veterinary medicine should therefore be assisted by pathologists, given the diversity of lesions and tumor types, as well as the variety of animal species encountered in routine diagnostic services (9).

For the analysis of a digitized image using AI, two main steps are required: identification of a region of interest and cellular analysis (1). Most tissue samples (biopsies, surgical specimens or necropsies) consist of a mixture of components: target tissue, blood vessels, stroma, tumor, and others. It is crucial to delineate the target compartment in the most relevant region, as the biomarker of interest may exist in other tissue compartments that are not pertinent to the study (1, 21). Once the region of interest is defined, the analysis algorithm is configured and tested to optimize precise cell analysis and parameter verification. Common user-configurable parameters include color, boundaries, and size (e.g., whether the algorithm expects a certain cell size, distinguishing adjacent or overlapping nuclei) (21). Optimizing image analysis algorithms is often an iterative process; the user adjusts parameters, runs the algorithm on representative images, evaluates and re-optimizes as necessary (11). To quantify the extent of cardiac or hepatic fibrosis, tissue sections can be stained with Masson's trichrome to highlight fibrotic areas, followed by quantification of the corresponding blue-stained area by an algorithm. For slides subjected to immunohistochemistry for cellular markers, measurements based on area or percentage of cells with positive immunolabeling can be applied. Then, algorithms can also be used to identify specific cell populations based on morphology and varying color intensities (22).

Despite its advantages and potential, the application of AI in DP is still limited due to its high cost and pitfalls in processing and training. In addition, it is crucial to develop appropriate quality control measures to assess potential artifacts throughout the workflow and image analysis. Therefore, the involvement of pathologists in the image analysis workflow is critical, including working collaboratively with software developers in constructing AI algorithms (21).

Data security

Traditional histopathology maintains privacy through physical limitations of sharing glass slides and the removal of patient identifiers (e.g., labels or erased information) (5). The generation of data from DP presents new challenges for the community in data management, processing, and security. This occurs because digital pathology data can be duplicated, are more mobile, and can be easily shared worldwide; therefore, additional measures are required to ensure patient data privacy (5, 6). Data security is therefore being extensively discussed in the human medical community. In veterinary medicine, patient's data such as species, breed, sex, and age are used to develop, train, and validate AI systems for histopathological examinations, and define prognostic indices. In some cases, this information is necessary for developing AI algorithms because some systems require large datasets for machine learning training and validation (5, 11). De-identification of digitized slides, the process of removing patient and owner information, should be carried out before sharing information with non-privileged parties or creating datasets for machine learning models or other applications (4, 11).

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Review of reports

A careful review of the quality control of the entire DP and AI process by a pathologist is essential (5, 8). Staining artifacts, over-segmentation or under-segmentation of nuclei and non-specific reactions in situ labeling techniques are among the issues that can compromise algorithmic image analysis (21). In this context, the pathologist should be able to identify and minimize technical artifacts. Regarding AI, pathologists trained in microscopic image examination should be involved in creating databases to ensure unbiased algorithm construction. If a dataset is imbalanced, such as predominantly comprising adult patients of a specific breed or sex, AI algorithm results trained on such data may not be accurate when implemented in the broader population.

Another issue relates to under specification, in which the AI training dataset lacks essential parameters. For instance, if population genetics are critical in histology image categorization, omitting these details will lead to an incompletely trained AI model. Under specification may consequently result in flawed correlations in outcome prediction (5). Ultimately, pathologists should be involved in reviewing reports whenever AI is used. Patients in human medicine and owners in veterinary medicine should be given the permission to decline decisions that are solely based on automated diagnosis.

Interpretation of lesions and results

Interpretations of microscopic findings are susceptible to biases due to subjectivity, which can be significantly reduced through pathologists' training and experience. However, complete elimination of this subjectivity is not possible. Biased analyses or interpretations can also be introduced during sampling, defining tissue analysis strategies, and constructing algorithms. Therefore, methodologies to minimize biases should be implemented in DP and AI with the involvement of a specialized stereologist (e.g., a pathologist with extensive experience in morphometry) to oversee study design, analytical approach, and data interpretation (22).

Teaching pathology

In education, many institutions have adopted DP for teaching histology and histopathology through digitized slides (19). Academic educational platforms with digitized slides enable international and interinstitutional collaboration between histologists and pathologists. Among the advantages of digitized slides for teaching are student access to the same slide, continuous access through an online repository, and the inclusion of image annotations to highlight specific resources for teaching (3, 19). Additionally, institutions do not need to purchase or maintain expensive microscopes for entire classes and sets of glass slides that require regular replacements of broken specimens. Likewise, pathology residency programs can utilize digitized slide sets for resident training on common histomorphologic features as well as on rare disease presentations. Additionally, veterinary pathology residents around the world heavily utilize open online resources maintained by the Davis-Thompson Foundation and the Joint Pathology Center (JPC), including the Veterinary Systemic Pathology Online and the weekly Wednesday Slide Conference, which provide access to digitized slides, descriptions and case notes from different countries around the world (22). Nonetheless, although young pathologists should be trained in the use of DP and applications of AI, the classical learning and training methods should not be replaced (11).

Conclusion

DP and AI have increasingly been applied in veterinary pathology as unique tools that streamline the diagnostic processes. Workflow based on digitized slides reduces travel, enhances communication and collaboration among pathologists, improves annotation capabilities, facilitates database construction with greater ease and accessibility, and increases specialist access, thereby allowing for quality histologic assessment. AI can relieve pathologists from laborious tasks such as mitotic counts, improved consistency in grading systems, and quantitative measurements, thereby supporting diagnostic decisions. However, these systems are costly and their use is still unfamiliar to some pathologists in Brazil. Moreover, integrating digital pathology with AI requires expertise in computational programs for developing accurate algorithms. Currently, there remains a degree of apprehension and skepticism surrounding the role of AI and its true impact on pathology. It is also important to acknowledge that AI-based technologies or machines cannot substitute pathologists, especially in postmortem diagnoses, for which all organs are examined histologically to determine the disease and/or cause of death. Instead of representing a replacement, these innovations therefore play an assistive role, enhancing pathologists' decision-making capacity and overall diagnostic performance.

Declaration of conflict of interest

The authors declare no competing interests.

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