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Novel computer-aided systems for interpreting immunohistochemistry (IHC) results in breast cancer based on deep learning algorithms: A systematic review

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ABSTRACT

Breast cancer is a prevalent disease worldwide and the accurate diagnosis and prognosis of breast cancer are essential for the development of effective treatment plans. Pathology remains the gold standard for diagnosis and prognosis but with limitations such as time-consuming manual scoring and some error-prone results. Recently, deep learning techniques, especially convolutional neural networks (CNN), have been proposed for the interpretation of immunohistochemistry (IHC) results in breast cancer. The objective of this systematic review is to critically assess the existing literature on computer-aided systems for the interpretation of IHC results in breast cancer based on deep learning algorithms. We included studies with models that use novel approaches such as deep learning for quantitative measurements of immunohistochemically stained Ki-67, ER, PR, and HER2 images. We systematically searched PubMed, Scopus, and web of science up to September 2022. 15 studies (seven HER2, seven Ki67, and one ER/PR scoring studies) met our inclusion criteria. Various AI-based assays have been developed for different applications in breast pathology, including diagnostic and prognostic applications, as well as predictive values and responses to treatment. These algorithms have shown promise in improving the accuracy of breast cancer diagnosis and prognosis. It is essential to consider the differences in training and inter-observer variability while designing tools, and there is an urgent need to integrate the detection and analysis of various biomarkers at the same place and time to facilitate the formation of patients' reports and treatment.

Keywords: breast cancer, deep learning, computer-aided systems, IHC.

INTRODUCTION:

Breast cancer is a heterogenous prevalent cancer which is one of the most common cancers among women around the world. This cancer treatment approach depends on its molecular characteristics (1). The Immunohistochemistry (IHC) diagnostic markers, such as estrogen receptor (ER), progesterone receptor (PR), Ki-67, and human epidermal growth factor receptor 2 (HER2), are widely used to identify metastatic and benign tumors, to grade tumors and to determine the origin of cancerous tissue. (2) Estrogen and progesterone are steroid hormones that play a crucial role in breast cancer development and pathology. They regulate the expression of several genes involved in cellular proliferation, tissue morphology, and other key biological processes. Consequently, breast carcinoma often exhibits altered expression of their receptors, in comparison to healthy tissue. The assessment of ER and PR expression is frequently employed to evaluate the response to hormone therapy and predict prognosis (3-6) HER2 protein, a cell membrane biomarker, is known as a diagnostic factor for breast cancer. Patients with breast tumors that overexpress HER2 have an aggressive type of disease and poor prognosis. (7-9) For cases with strongly overexpression of HER2, the addition of targeted treatment against HER2 is particularly effective at improving clinical outcome compared to chemotherapy alone. (10) HER-2 positive tumors can be more aggressive and their status can predict the response to targeting therapy with trastuzumab (Herceptin) monoclonal antibodies and adjuvant chemotherapy. (11) Therefore, the correct identification of the HER2 is critical to help patients to receive the appropriate therapeutic option.

The Ki-67, also called MKI67, is a nuclear protein associated with cell proliferation (12) Ki-67 helps detect proliferating cells in the colonic epithelium in ulcerative colitis (13) some brain tumors (14) non-Hodgkin lymphomas (15), lung cancer (16), and breast lesions. (17) Immunohistochemical analysis of Ki-67 is a clinical marker for breast cancer tumor aggressiveness and proliferation. This assessment helps predict disease survival, and recurrence, deciding the future course of therapy, and response to various treatment options. Therefore, the scoring of Ki-67 is highly relevant for the diagnosis, classification, prognosis, and treatment. In addition, it helps predict relative responsiveness or resistance to chemotherapy or endocrine therapy. Ki-67 scoring is also valuable for estimating residual risk in patients on standard therapy. Moreover, it is a dynamic biomarker of treatment efficacy in samples taken before, during, and after neoadjuvant therapy, particularly neoadjuvant endocrine therapy. (18)

The advances in biomarker technology allow us to use the most appropriate treatment and procedure according to the subtypes of the disease.

Digital pathology is the process of digitizing and computerizing tissue sample slides using a whole slide image (WSI) scanner and then analyzing or sharing the digital images using image viewer, on electronic devices. The patients who would benefit from genomic testing the most could be found by image analysis of hematoxylin and eosin or H&E-stained images. Several previous studies have utilized automated processing of toxin H&E-stained breast tumor slides for diagnosis, prognosis, and feature identification associated with survival. These approaches have focused on statistics of cell morphology (19), automated grading, mitotic count (20), nuclear atypia, receptor status, and histopathological subtyping (21) using hand-crafted methods or automated models.

Moreover, several factors such as staining, orientation, and magnification of the biological sample contribute to several issues such as the visual heterogeneity of the images, illumination variations, foreground-background intensity overlaps, partial occlusion, and weak boundaries. These issues affect the morphological structure of the histological regions and due to them, more consideration during image processing and training is required (22).

Breast cancer subtyping using biomarker assessment, play a crucial role in determining an appropriate treatment plan. To assess protein expression at the

tissue level, molecular markers with both prognostic and diagnostic value are used as indicators. IHC is employed as the primary method for staining these biomarkers. A pathologist then examines the stained tumor tissue under a microscope and assigns a score based on the percentage of positively stained cell nuclei (23). This manual scoring process, however, can be time-consuming, tedious, expensive, error-prone, and susceptible to intra- and interobserver ambiguities that lead to inconsistent scores. (24) This shows that there is a challenge of repeatability in manual scoring.

Recently, deep learning techniques have dramatically advanced and have been assessed in scoring predictive marker such as ER, PR, HER-2, Ki-67 proliferation, and intrinsic subtype assessment. Among deep learning models, convolutional neural networks (CNN) have received the most attention and validation (22).This study aims to conduct a systematic review of computer vision models for the automated scoring of predictive markers, such as ER, PR, and HER-2, and to update the existing evidence for the development of future work.

Method and material:

Eligibility criteria

Eligible studies were those published which reported a computer vision model to indicate receptor status and assess the intrinsic subtype of breast cancer histopathological samples. We included models that use novel approaches such as deep learning for quantitative measurements of IHC stained Ki-67, ER, PR, and HER2 images. We excluded the review papers and publications that were based on non-human or generated images with novel data generating methods such as generative adversarial networks (GANs) and also the publication that only used models for the analysis of H&E-images. Also Additionally, traditional models which indicated the score of staining pictures that only considered the intensity of color were excluded.

Data sources and searches

A search was conducted across three databases (PubMed, Web of Science, and Scopus) to identify artificial intelligence models that predict the scoring of biomarkers based on histopathological images using deep learning approaches.

We searched PubMed using the terms “Artificial intelligence”, “Breast Neoplasms” [MeSH term], “Pathology” [MeSH term], OR “Neoplasms/pathology”, in combination with related key terms such as “patholo*”, “histopatholo*, “microscop*”, “digital*”, “whole-slide”, “deep learning”, etc. in the title and abstract. Entry terms were used to search Web of Science and Scopus databases. All The searches were updated in September 2022. No language limitation was applied. However, all found publications were in English.

Study selection and Data extraction

The screening of the search results was conducted by two reviewers independently who evaluated the titles and abstracts of the studies. To confirm the eligibility of the relevant studies, two reviewers independently reviewed the full text articles. In case of researchers’ disagreements, the inclusion of studies was determined by consensus. PRISMA flowchart (Figure. 1) shows the result of the extraction process. Predefined tables have been used for extraction of the following items from included studies: author, publication date, type of biomarkers, staining approaches, the name of the model if available, sample characteristics, sample size, type of breast cancer, artificial intelligence, or deep learning algorithms. Data abstraction was conducted by one reviewer and checked by another to ensure accuracy.

Result:

Study inclusion

A total of 1811 articles were found through dataset primary searches. After removing duplicates, 1429 unique records were screened. Of these, 1376 studies were excluded at the title and abstract evaluation phase. Finally, 53 articles were screened for full-text (Figure 1).

Among 53 potentially relevant full-text screened articles, 15 studies met the inclusion criteria. Exclusion reasons are outlined in the PRISMA flowchart (Figure 1). The complete table of developed models is provided in Table 1.

Study characteristics

Among 15 included studies, seven studies evaluate HER2 scoring, seven studies evaluate Ki67 scoring, and one study evaluates ER and PR scoring.

Estrogen receptor (ER) and progesterone receptor (PR)

Saha et al. (25) proposed HscoreNet, a unique deep learning network for scoring ER and PR in breast IHC images. The network can be used for semantic segmentation, classification, and picture reconstruction in addition to scoring. This network is divided into three sections: encoder, decoder, and scoring layer. The encoder converts input image pixels into a lower-dimensional representation, whereas the decoder reconstructs the encoder's output by minimizing a cost function. The decoder produces the reconstructed image, which only comprises immunopositive and immunonegative nuclei. The final layer is a scoring layer, which is in charge of calculating the H-score.

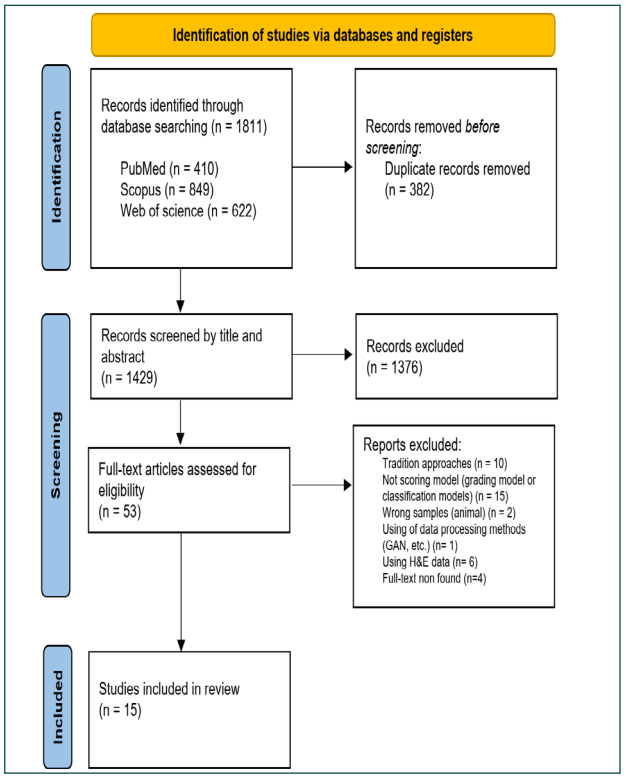

Figure 1. PRISMA flow chart of selected studies

Human epidermal growth factor receptor 2 (HER-2)

Khameneh et al. (26) proposed a method that combines deep learning and conventional machine learning techniques to segment, classify, and quantify IHC images of breast cancer. This method involves two main steps: segmentation and classification. Since HER2 is mainly related to tumors of the epithelial region, the authors segmented different tissue structures to identify epithelial regions. To achieve this, they used a superpixel-based support vector machine (SVM) feature learning classifier to classify stromal and epithelial regions from WSIs based on color and texture features. Next, they applied a CNN based segmentation method (modified U-Net model) on the epithelial regions to segment membrane regions. Finally, the authors merged divided tiles and evaluated the overall score of each slide. The proposed method was compared to other approaches based on deep learning and handcrafted features on 127 WSIs of breast tumor patients. The results showed that the proposed approach had promising performance on IHC stained slides.

Tewary and Mukhopadhyay (27) used several transfer learning architectures for HER2 scoring and classification, including VGG16, ResNet50, VGG19, NASNetMobile, and MobileNetV2, to categorize images into three output classes: negative, equivocal, and positive.. They used 2130 patches for generating training dataset, then tested the output model for 800 new patches to report outcome result. They also used a voting scheme to generate an overall score for an image using the scores of its patches. These models have shown noteworthy accuracy and VGG19 had the most accuracy between them.

Vandenberghe et al. (28) proposed two distinct approaches for cell classification, using both classical machine learning and deep learning techniques. Their

deep learning approach enabled automatic scoring of HER2, with an 83% concordance rate with pathologist scores. Diagnostic disagreements were largely attributed to discrepancies in HER2 expression due to high staining heterogeneity. They demonstrated that deep learning assisted diagnosis can improve clinical decision-making in breast cancer by identifying high-risk cases of misdiagnosis (7).

Tewary et al. (29) have developed an automated scoring system for detecting HER2 from stained tissue images called AutoIHC-Analyzer. They have used several image-processing approaches to extract the stained cells and membrane regions automatically. At last, they used the set of features to classify the tissue for quantitative scoring. The results show reliable quantification for automated scoring. Although, they used a complicated algorithm with a number of image processing steps in the assessment of stains followed by morphology-based quantification of membrane continuity and automated scoring using machine learning (SVM classifier).

In another study, Yue et al. (30), an artificial intelligence (AI)–assisted microscope was equipped with a conventional microscope with a cell-level classification-based HER2 scoring algorithm and an augmented reality module. The consistency and accuracy of HER2 assessment were significantly improved ($p < 0.001$) in comparison to using a conventional microscope and online WSI. Furthermore, results showed improved precision of IHC 3 + and 2 + scoring while ensuring the recall of fluorescent in situ hybridization (FISH)–positive results in IHC 2 +. Also, the average acceptance rate of AI (LinkNet (31) with mean square error (MSE) loss for nucleus detection) for all pathologists was 0.90, demonstrating that the pathologists agreed with most AI scoring results.

Her2Net (2), a deep learning-based HER2 deep neural network, consisted of multiple convolution layers, max-pooling layers, spatial pyramid pooling layers, deconvolution layers, up-sampling layers, and trapezoidal long short-term memory (TLSTM). It was applied for cell membrane and nucleus detection, segmentation, classification and HER2 scoring by using TLSTM and a deep learning framework. Her2Net achieved 96.64% precision, 96.79% recall, 96.71% F-score, 93.08% negative predictive value, 98.33% accuracy, and a 6.84% false-positive rate. The results showed the high accuracy and wide applicability of Her2Net in HER2 scoring for breast cancer assessment.

Wang et al. (32) proposed a multitask CNN to identify magnification and the score of HER2 expression. In addition, they compare its accuracy, precision, and recall with another neural network like Lenet, AlexNet, and Vgg16. The results have shown in Table 2 and 3. According to the results, the proposed network is

Table 2. results of each network in HER2 scores.

network	Accuracy [%]	Precision [%]	Recall [%]
Lenet	0.871	0.878	0.871
AlexNet	0.938	0.939	0.938
Vgg16	0.915	0.916	0.915
Wang et al. [31]	0.978	0.977	0.978

Table 3. results of each network in HER2 magnification.

network	Accuracy [%]	Precision [%]	Recall [%]
Lenet	0.842	0.854	0.842
AlexNet	0.942	0.943	0.942
Vgg16	0.925	0.926	0.925
Wang et al. [31]	0.950	0.950	0.950

lighter than the others and costs much less time than the other mature networks in training.

Proliferation of Ki-67

Joseph et al. (33) developed Proliferation Tumor Marker Network (PTM-NET). This deep learning model objectively annotates the tumor regions in Ki67-labelled breast cancer digital pathology whole slide images using a convolution neural network.

Feng et al. (34) introduced an accurate image registration method and automatic identification and counting software of Ki-67 based on whole tissue sections by deep learning. This method was designed with unsupervised domain adaptation for counting. (Based on GoogLeNet Inception V1)

Swiderska-Chadaj et al. (35) presented three deep learning-based approaches (CNN) to automatically detect and quantify Ki-67 hotspot areas utilizing the Ki-67 labeling index in whole slide images. All processes were based on Deep Learning CNNs (using the AlexNet model) and were developed to explore the possibilities of combining information from several stains.

Geread et al. (36) proposed a novel proliferation index (PI) calculator for Ki67 images called piNET. The tool is built based on deep learning, which can adapt to the wide variability of medical images. The system is trained purely on tumor cells, which reduces false positives from non-tumor cells. In addition, the concept of learning background regions through weak supervision is introduced ~~and~~ which provides the system with ideal and non-ideal patches that further reduce false positives. Also, a novel hotspot analysis is proposed that allows automated methods to only score patches from the WSI that contain "significant" activity. One of the significant contributions of the architecture's overall robustness is that the model can quantify Ki67 PI for various image types (regions of interest (ROIs), Tissue Microarrays (TMAs), WSIs), scanner/ stain vendors, lab staining protocols, and the presence of artifacts or non-tumor cells. (36)

Cai et al. (37) presented an algorithm with three steps for Ki67 computation. At first, Fully convolutional network (FCN) LinkNet was used for tumor region segmentation. Second, another FCN model was used in segmented tumor region for generating nuclear heatmap and a local maximum filter was utilized to calculate the nuclear centers. Third, the DAB color space was used to distinguish between positively stained and negatively stained nuclei.

Negahbani et al. (38) presented a unique pipeline and backend for the simultaneous assessment of the intratumoral TILs score and estimation of Ki-67 expression in breast cancer cells. This pipeline uses CNN to estimate density maps and extract features from an input RGB image. This pipeline consists of three parts; PathoNet network, post-processing, and Watershed algorithm. PathoNet gather features from input images and then predicts the pixels for being either immunopositive or immuninegetive for Ki67.

Fulawka et al. (39) proposed a solution computes the Ki-67 proliferation index using a deep learning model and fuzzy-set interpretations for detecting hotspots. The resultant region-of-interest is then utilized to segment relevant cells using traditional image processing methods. The index value is calculated by comparing the total surface area of immunopositive cells to the total surface area of relevant cells.

Discussion:

Breast cancer is one of the most common cancers among women worldwide, and accurate diagnosis and prognostic evaluation are critical for determining appropriate treatment strategies. Traditionally, the evaluation of breast cancer involves manual observation of stained tissue samples under a microscope by expert pathologists. However, this process is time-consuming, prone to inter- and intra-observer variability, and often results in inaccurate and irreproducible diagnostic and prognostic evaluations. In recent years, artificial intelligence has emerged as a promising tool for improving the accuracy and efficiency of breast cancer pathology.(40)

AI techniques can be used to analyze digital pathology images and extract meaningful information that can

assist in the diagnosis and prognosis of breast cancer. One of the most commonly used AI techniques in breast cancer pathology is machine learning, which involves training models on large datasets of annotated images to recognize patterns and make predictions. machine learning algorithms can be trained to identify specific features of breast cancer tissue, such as the presence of estrogen and progesterone receptor expression, which are important markers for determining treatment strategies. (41)

In addition to predicting markers for breast cancer diagnosis, AI techniques can also be used to predict patient outcomes and personalize treatment strategies. For example, a study by Beck et al.(42) developed a machine learning model to predict the risk of recurrence in breast cancer patients based on tumor morphology and other clinical data. The model achieved high accuracy in predicting recurrence risk on a dataset of over 3,000 patients.

Despite the potential benefits of AI in breast cancer pathology, there are also several challenges that need to be addressed. One of the biggest challenges is the lack of standardized protocols for data collection and analysis. Different laboratories may use different staining protocols and imaging systems, which can lead to variability in the data and make it difficult to compare results across studies. To address this challenge, several initiatives have been launched to develop standardized protocols for digital pathology, such as the Digital Pathology Association's Image Analysis Standards Initiative (43).

Another challenge is the lack of diversity in the datasets used to train AI models. Many studies use datasets from a single institution or geographic region, which may not be representative of the broader population. To address this challenge, several initiatives have been launched to develop large-scale, diverse datasets for AI research, such as the Cancer Imaging Archive (44).

The generation of digital pathology data creates novel challenges for the histopathology community in managing, processing, and controlling the use of these data. The legal and ethical aspects of digital pathology, for example, the pathologists' interpretation of consent for scanned slide images in research, are unclear. Digital pathology is not free of ethical challenges. It may involve sharing sensitive personal data if it needs collaboration, which is subject to specific ethical and legal norms. Due to the black box problem, AI methods are mysterious and produce results that are sometimes unexplainable even to experts. Computational pathology mainly depends on scanning technology manufacturers who benefit from data collection. Digital data is subject to fusion or analytics, often without the knowledge of people who have consented to its collection. Fusion and analytics are not always subject to formal surveillance. Once data are in digital form, there is often confusion as to who should decide its reuse and which reuses are legitimate. (45, 46) To address these challenges, several studies have proposed frameworks for the ethical and responsible use of AI in breast cancer pathology. For example, a study by Gurcan et al. (47) proposed a framework for the validation of AI algorithms in digital pathology, which includes guidelines for data collection, annotation, and evaluation.

In addition to these challenges, there are also technical challenges in the use of AI in breast cancer pathology. The effect of the training set size and the depth of experience of the operator annotating the training set on the digital pathology mode. It could provide valuable information to optimize accuracy. (28)

Another technical challenge is the variability in staining and imaging protocols, which can lead to differences in the appearance of tissue samples and make it difficult to compare results across studies. Various staining criteria of different laboratories may cause discordant results. This discrepancy was the main reason for misclassifications. Overlapping of cytoplasmic staining with cell membrane gives rise to poor segmentation, which causes errors in a way that some membrane staining connecting two cells is ignored, which would directly affect the overall score in some cases. (26) To address this challenge, several studies have proposed methods for normalizing digital pathology images, such as the use of color normalization techniques to

standardize the color appearance of images .(48)

Various staining criteria of different laboratories may cause discordant results. This discrepancy was the main reason for misclassifications. A better and more accurate histopathology stain-color normalization may overcome this problem. Overlapping of cytoplasmic staining with cell membrane gives rise to poor segmentation, which causes errors in a way that some membrane staining connecting two cells is ignored, which would directly affect the overall score in some cases. (26)

Factors that lead to poor reproducibility of scoring results may include the type of biopsy, time to fixative, type of antibody, method of reading, and area of reading. between-laboratory and between-study comparability is essential. (34) However, more statistical analysis could be performed with a wider sample size and the increment of training data from various laboratories to validate the approach. Further improvement in terms of sample variability and broader dataset could be targeted in the future. (27)

Several suitable types of AI algorithms in breast pathology are divided into three major groups: diagnostic, prognostic applications, and applications related to predictive values and response to treatment. The prognostic applications include determining tumor morphological characteristics, such as nuclear shape and texture, using in survival and recurrence risk prediction, and various peri-tumoral elements involved in prognosis. (22) Different AI-based assays have been developed to measure the architecture of various tissue elements, including tumor-infiltrating lymphocytic (TIL) within the tumor, and their efficacy in predicting survival has been established. (49) As a second major application of AI in breast pathology, machine learning approaches can be used to link the expression of specific markers, such as cell cycle and proliferation markers, or the presence of specific morphological features in the tumor to the response to a particular treatment. (50) The diagnostic applications include breast cancer grading and intrinsic subtype detection, tumor microenvironment, receptor status and heterogeneity assessment, and metastatic tumor deposit detection in lymph nodes. (22) Several algorithms assess breast cancer grades by pattern recognition analysis using deep learning (21) or allowing the accurate counting of mitotic figures, which is one of the essential features of grading. (51) Image analysis techniques have been used for detecting histologic subtypes of breast cancer or classification. (21) Many AI-based assays have been developed to evaluate intra-tumor and inter-tumor heterogeneity (51), identify and quantify nonepithelial cells like fibroblasts, neutrophils, lymphocytes, and macrophages (49), and automated image-based identification and grading of TIL in HER2+ breast cancer. (50) One of the new applications of diagnostic pathology is quantitative measurements of IHC-stained ER, Ki67, PR, and HER2 images (22), which is extensively discussed in this study.

Different medical and pathological groups at different locations communicate with each other to discuss and put in efforts to analyze the results. Differences in training and inter-observer variability are also factors that must be taken into consideration while designing tools. There is an urgent need to design algorithms that address the mentioned challenges. The nuclei texture properties or other characteristics could be used as prior information in algorithms to make the tool more robust. Breast cancer diagnosis includes the detection of ER, PR, Ki-69, and HER2 receptors in stained tissue. Integration of detection and analysis of these procedures at the same place and time would greatly help pathologists and doctors to form patients' reports and treatments. (52)

In this line, some digital pathology companies have developed different image analysis platforms for tissue classification, biomarker analysis, IHC quantification, and molecular pathology. IHC biomarker detection and immune-oncology biomarker analysis are the primary categories on which these businesses focus. For IHC biomarker detection, the cytonuclear IHC and membrane IHC modules are particularly challenging. The cytonuclear IHC module measures IHC positivity cell-by-cell for single or multiple staining applications.

Each cell is assessed for the cytoplasmic or nuclear positive of every single stain, as well as the colocalization of several marker combinations. This module can be utilized for the detection of particular biomarkers such as ER, PR, and Ki67. Different companies, including 3DHISTECH, Roche, Indica Labs, and Visiopharm, provide specific platforms for detecting ER, PR, and Ki67. NuclearQuant is a software application developed by 3DHISTECH company, which achieved in vitro diagnostics (IVD) approval for the analysis of Estrogen- and Progesterone-stained breast tissue samples. CellQuant is another image analysis tool of this company that is best suited for Ki67 slides. uPath is a sub-organization of Roche company that provides various image analysis algorithms, and detection of ER, PR, and Ki67 is one of its primary objectives. As a part of Indica Labs company, HALO provides the cytonuclear IHC module, which can be used to detect similar biomarkers.

The membrane IHC module performs cell-by-cell analysis of the levels of membrane-associated IHC markers. This color-configurable module can be utilized with non-traditional chromogens in addition to DAB. This module also can be utilized for the detection of particular biomarkers such as HER2, which is provided by 3DHISTECH, Roche, and Indica Labs commercially. MembraneQuant, like NuclearQuant, is an IVD-approved software application that has been designed for HER2 expression quantification in breast tissue samples. One of the other primary objectives of uPath software is HER2 detection. Same as cytonuclear IHC, HALO provides the membrane IHC module for HER2 detection. Visiopharm, a privately-owned company founded in 2002, is one of the institutes that provides more than 100 special apps in the field of diagnosis and research for the detection of variable biomarkers, including both cytonuclear and membrane IHC, In addition to hot spot detection.

For the immune-oncology biomarker analysis, PD-L1 expression and CD8 positivity quantification are regarded as new modules in the field of digital pathology. The presence of CD8+ T cells in the tumor microenvironment is associated with response to immunotherapy and can inform patient treatment decisions. Also, the development of the combined positive score (CPS) for the evaluation of PD-L1 in solid tumors has indicated that PD-L1 expression on both tumor and tumor-associated immune cells is associated with clinical outcomes. Some companies such as PathAI, Nikon Cooperation, and Roche provide various algorithms for these marker quantification analyses. PathAI is currently developing machine learning-based models to identify and quantify CD8+ lymphocytes within the stroma and parenchyma regions of tumors from other cancers, including non-small cell lung cancer, renal cell carcinoma, breast cancer, gastric cancer, head and neck squamous cell carcinoma, and urothelial carcinoma. Also, it provides PD-L1 expression analysis for this aim. Optra scan, a part of Nikon Cooperation, and Roche are also working on the PD-L1 expression analysis.

Some companies are working on other software and modules, including visiopharm, indica Labs, for automated or Add-on's quantification, which are not discussed in this article and require more studies.

Further case details in the last stage of the pipeline, e.g., the other IHC results and patient data, could be included. In this way, even more information could be provided to the medical experts to provide a robust tool that can be practically used in clinical decision-making. (53) Future work is directed towards a more exhaustive analysis of the feature vectors with variations in examples using a much larger number of image tiles extracted from WSIs for training and cross-validation. Further work will also be carried out in developing and comparing different training for neural network modeling to improve histology slides' automatic scoring. (54)

Conclusion:

In conclusion, AI techniques have revolutionized the field of pathology by automating the diagnosis and prognosis of cancer. However, the use of digital pathology data presents novel challenges in terms of

data management, ethical and legal considerations, and inter- and intra-observer variability. There is a need to address these challenges through the development of robust algorithms that take into account the effect of the size of the training set and the depth of experience of the operator annotating it. Various AI-based assays have been developed for different applications in breast pathology, including diagnostic and prognostic applications, as well as predictive values and responses to treatment. These algorithms have shown promise in improving the accuracy of breast cancer diagnosis and prognosis. It is essential to consider the differences in training and inter-observer variability while designing tools, and there is an urgent need to integrate the detection and analysis of various biomarkers at the same place and time to facilitate the formation of patients' reports and treatment. Several digital pathology companies have developed different image analysis platforms for tissue classification, biomarker analysis, and molecular pathology, focusing on IHC biomarker detection and immune-oncology biomarker analysis. In order to maximize the potential of AI in pathology, it is crucial to continue to address the challenges and improve the accuracy of these algorithms.

Author contributions:

Sasan Salehi Nezamabadi and Golnar Khorsand designed the research. Sasan Salehi Nezamabadi, Elham Angouraj Taghavi, Golnar Khorsand, and Parsa Mirzayi conducted the search, screening, and data extraction. Sasan Salehi Nezamabadi and Haniyeh Rafiepoor wrote the draft of this paper. Sasan Salehi Nezamabadi, Haniyeh Rafiepoor, Mohammad Amin Barati, Ali Taheri, Behzad Amanpour-Gharaei, Seyed-Ali Sadegh-Zadeh, Saeid Amanpour revised the article critically. All the authors contributed to the manuscript writing, read and approved the final manuscript. This paper had conducted under supervision of Seyed-Ali Sadegh-Zadeh and Saeid Amanpour.

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The authors declare that there is no conflict of interests.

Table 1. Summary of included studies

	Topic	Digital pathology model	Breast cancer sub-type	Task	Target population	sample size	c-statistic/AUC accuracy	Sensitivity*	Precision	F1 score	AI algorithms
1	HER2 status	Khameneh et al. 2019 (26)	All types	1)classification of WSI into two stroma and epithelium areas using superpixel-based classification 2)Cell membrane staining pattern extraction 3) HER2 score assessment	Breast tumor patients from Acıbadem hospital and Warwick competition	127 WSIs	94.82% segmentation and 87% classification accuracy	99.70% segmentation	92.83% segmentation	95.80% segmentation	CNN (DenseNet, SegNet, M odified U-Net) k-mean, SVM
2	HER2 Patch based scoring	Tewary et al. 2021 (27)	All types	HER2 Quantification and automated scoring using transfer learning followed by statistical voting	HER2 Challenge dataset from Warwick University	2130 training patches (300 images, 30 cases) and 800 test patches (100 images, 10 cases)	VGG19—patch-based scoring: 93% VGG19—image-based scoring with voting: 98%	VGG19—patch-based scoring: 92.3% VGG19—image-based scoring with voting: 98%	VGG19—patch-based scoring: 93% VGG19—image-based scoring with voting: 98.3%	VGG19—patch-based scoring: 92.7% VGG19—image-based scoring with voting: 98%	CNN (VGG16, VGG19, ResNet50, MobileNetV2, and NASNetMobile)
3	extraction/ scoring in HER2	Tewary et al. 2020 (AutoIHC-Analyzer) (29)	Invasive breast cancer	Automated HER2 cell membrane extraction, quantification of continuity of cell membrane, scoring	Tata Medical Center (TMC), Kolkata, India	180 training images 90 validations	Accuracy 93% Macro accuracy 94% Correlation coefficient	93.6%	94%	94%	CNN (SVM with Gaussian kernel)
4	scoring of HER2	Vandenberghe et al. 2017 (28)	Invasive carcinoma	Cell detection and classification and automatic scoring of HER2	AstraZeneca BioBank	74 WSI (71 slides were selected)	83 % () Cohen's κ = 0.69 Kendall's τ = 0.84 10-fold cross validation : 78 %	-	-	72.5 %	CNN (ConvNet)
5	scoring of HER2	Wang et al. 2018 (32)	All types	Automatic HER2 scoring and magnification	Not mentioned	10 WSI (12000 training patches, 4800 validation patches, 20000 test patches)	Scoring: 97.8 % Magnification: 95.0 %	-	Scoring: 97.7% Magnification 95.0%	Scoring: 97.8% Magnification 95.0%	CNN (LeNet, AlexNet, Vgg16) Data preparation: SVM, label propagation (LPA)
6	scoring of HER2	Yue et al. 2021 (30)	Infiltrating duct carcinoma	Tumor cell detection and classification, membrane delineating Improvement of the HER2 assessment accuracy and reliability	Cases of postoperative infiltrating duct carcinoma not otherwise specified (NOS) and without neoadjuvant treatment from the Fourth Hospital of Hebei Medical University	50 WSI (25 HER2 positive, 25 HER2 negative)	Cohen's kappa: κ = 0.86 [95% CI 0.84–0.89]	-	-	-	Fully convolutional network (FCN) for tumor cell detection and classification LinkNet for nuclear detection

7	scoring of HER2	Saha et al. 2018 (Her2Net) (2)	Invasive breast carcinomas	Identification, segmentation and classification of cell membranes and nuclei from HER-stained breast cancer images.	online HER2 image database of the Department of Computer Science, University of Warwick, United Kingdom.	79 WSI (training: 51, testing: 28, 752 images)	98.3 %	96.8 %	96.6 %	96.7 %	CNN (fully connected TLSTM, SegNet, Bayesian SegNet, U-Net)
8	Scoring of Ki67	Joseph et al. 2019 Proliferation Tumour Marker Network (PTM-NET) (33)	Invasive ductal carcinoma	Identification of tumor region, Identification of high density Ki67	30 Ki67-labelled images from the AstraZeneca (AZ) validation cohort 15 Ki67-labelled images from the Vilnius University (VU) cohort	102 WSIs (Training: 45 Testing: 12 Validation: 45)	Dice score: PTM-NET: 77 % VGG-NET: 69 %	-	-	PTM-NET: 93.5 % VGG-NET: 91.9 %	CNN (VGG16, PTM-NET)
9	Scoring of Ki67	Feng et al. 2020 (34)	invasive ductal carcinoma	Automated quantitative analysis of Ki-67, Ki-67 Automatic Counting Software in breast IDC on WSI	IDC slides from Department of Pathology of West China Hospital Sichuan University, China	WSI training: 677 patch no. 11,628,208 verification: 153 Patch no. 2,973,384 test: 187 Patch no. 2,419,032 Sum 1017 WSI Patch no. 17,020,624	Accuracy: 89.44% Balance accuracy: 90.14 %	85.05%	82.86%	-	CNN (GoogleNet)
10	Scoring of Ki67	Cai et al. (2021) (37)	invasive ductal carcinoma	Improving Ki67 Assessment Concordance with AI-Empowered Microscope	cases of Invasive breast cancer Fourth Hospital of Hebei Medical University	Training set: 632 WSI from 3 hospitals 5920 patches from mentioned WSIs	AI-empowered Microscope + experienced pathologist (ICC = 0.937) inexperienced pathologists (ICC = 0.923)	-	-	83.1 %	FCN (LinkNet, ResNet18 as backbone)
11	Scoring of Ki67	Swiderska-Chadaj et al. (2020) (35)	invasive breast cancer	Detection of Ki67 Hot-Spots of Invasive Breast Cancer	Digital slides from the AIDPATH breast cancer database, breast tissue cohorts from four institutions and pathology labs, Europe (Spain, Italy and Lithuania)	100 whole slide images (WSIs) belonging to 50 breast cancer patients	Overall accuracy: 95% Method1:96% Method 2:95% Method 3: 95% Spearman's p correlation of 0.92	Method1: 47% Method 2: 39% Method 3: 77%	Method1:47% Method 2:44% Method 3: 70%	Method1:47% Method 2:41% Method 3:73%	Method1: Ki67 data (1 CNN, AlexNet) Method 2: Ki67 and H&E data (2 CNNs) Method 3: Ki67 and H&E data (color deconvolution + 1 CNN)
12	Scoring of Ki67	Geread et al. (2021) (pi-NET) (36)	Invasive breast cancer	Fully automatic Ki67 Proliferation Index Calculator	St.Michael's Hospital, Toronto, Canada, Protein Atlas, Deep Slides, Ontario Veterinary College at the University of Guelph	55 WSI, 773 patches (ROI), and 90 TMAs	Overall accuracy: 85.2% ROIs: 87.9% TMAs: 85.5% WSI: 76.4%	-	-	Ki67−: 78.8% Ki67+: 82.0%	pi-NET other deep learning methods (ResUNet, DenseUNet, FCN8) unsupervised method (IHCCH)

13	Scoring of Ki67	Negahbani et al. (2021) (PathoNet) (38)	Invasive ductal carcinoma	Evaluation of Ki-67 and tumor-infiltrating lymphocytes in breast cancer	Shiraz Histopathological Imaging Data Center	23 cases (1656 training and 701 test image)	95.6%	86.1%	84.4%	85.2%	PathoNet, FCRN-A, FCRN-B, Mobilenet, Xeption, Modified U-Net
14	Scoring of Ki67	Fulawka et al. (2022) (39)	ductal carcinoma in situ	Assessment of Ki-67 proliferation index with deep learning in DCIS	Lower Silesian Oncology Center, Wroclaw Medical University, Poland	95 WSI	83.7%	75.1%	82.4	74.1%	DenseNet121
15	ER and PR scoring	Saha et al. (2020) (HscoreNet) (25)	Invasive breast cancer	Automated scoring of ER and PR, semantic segmentation, classification, and image reconstruction	Tata Medical Centre (TMC), Kolkata, Indian Institute of Technology, Kharagpur, India	600 images (300 ER and 300 PR) 359,884 (231,851 immune positive and 128,033 Immune negative) nuclei	94.53%	95.64%	95.87%	96.49%	HscoreNet

*Best sensitivity, specificity, accuracy and precision reported in this article
WSI: whole-slide image, MRF: Markov random field, IHC: Immunohistochemistry, ROI: regions of interest, TMA: tissue microarrays. CNN: convolutional neural network, DCIS: ductal carcinoma in situ

REFERENCES:

1. Barzaman K, Karami J, Zarei Z, Hosseinzadeh A, Kazemi MH, Moradi-Kalbolandi S, et al. Breast cancer: Biology, biomarkers, and treatments. International Immunopharmacology. 2020;84:106535.
2. Saha M, Chakraborty C. Her2Net: A Deep Framework for Semantic Segmentation and Classification of Cell Membranes and Nuclei in Breast Cancer Evaluation. IEEE Trans Image Process. 2018;27(5):2189-200.
3. Jensen EV, editor Mechanism of estrogen action in relation to carcinogenesis. Proceedings Canadian Cancer Conference; 1966.
4. Kraus JA, Dabbs DJ, Beriwal S, Bhargava R. Semi-quantitative immunohistochemical assay versus oncotype DX® qRT-PCR assay for estrogen and progesterone receptors: an independent quality assurance study. Modern Pathology. 2012;25(6):869-76.
5. Joensuu K, Leidenius M, Kero M, Andersson LC, Horwitz KB, Heikkilä P. ER, PR, HER2, Ki-67 and CK5 in Early and Late Relapsing Breast Cancer-Reduced CK5 Expression in Metastases. Breast Cancer (Auckl). 2013;7:23-34.
6. Iqbal B, Buch A. Hormone receptor (ER, PR, HER2/neu) status and proliferation index (Ki-67 marker) in breast cancers: Their Onco-Pathological Correlation, shortcomings and future trends. Med journal of Dr D Y Patil university. 2016;9:674-9.
7. Hicks DG, Schiffhauer L. Standardized Assessment of the HER2 Status in Breast Cancer by Immunohistochemistry. Laboratory Medicine. 2011;42(8):459-67.
8. Tabakov M, Kozak P. Segmentation of histopathology HER2/neu images with fuzzy decision tree and Takagi–Sugeno reasoning. Computers in Biology and Medicine. 2014;49:19-29.
9. Rakha EA, Pinder SE, Bartlett JM, Ibrahim M, Starczynski J, Carder PJ, et al. Updated UK Recommendations for HER2 assessment in breast cancer. J Clin Pathol. 2015;68(2):93-9.
10. Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. Nat Rev Clin Oncol. 2011;9(1):16-32.
11. Ross JS, Fletcher JA, Linette GP, Stec J, Clark E, Ayers M, et al. The Her-2/neu gene and protein in breast cancer 2003: biomarker and target of therapy. Oncologist. 2003;8(4):307-25.
12. Schonk DM, Kuijpers HJ, van Drunen E, van Dalen CH, Geurts van Kessel AH, Verheijen R, et al. Assignment of the gene(s) involved in the expression of the proliferation-related Ki-67 antigen to human chromosome 10. Hum Genet. 1989;83(3):297-9.
13. Andersen SN, Rognum TO, Bakka A, Clausen OP. Ki-67: a useful marker for the evaluation of dysplasia in ulcerative colitis. Mol Pathol. 1998;51(6):327-32.
14. Giangaspero F, Doglioni C, Rivano MT, Pileri S, Gerdes J, Stein H. Growth fraction in human brain tumors defined by the monoclonal antibody Ki-67. Acta Neuropathologica. 1987;74(2):179-82.
15. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol. 1984;133(4):1710-5.
16. Martin B, Paesmans M, Mascaux C, Berghmans T, Lothaire P, Meert AP, et al. Ki-67 expression and patients survival in lung cancer: systematic review of the literature with meta-analysis. Br J Cancer. 2004;91(12):2018-25.
17. Elkablawy MA, Albasri AM, Mohammed RA, Hussainy AS, Nouh MM, Alhujaily AS. Ki67 expression in breast cancer. Correlation with prognostic markers and clinicopathological parameters in Saudi patients. Saudi Med J. 2016;37(2):137-41.
18. Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, A'Hern R, et al. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. J Natl Cancer Inst. 2007;99(2):167-70.
19. Beck AH, Sangoi AR, Leung S, Marinelli RJ, Nielsen TO, van de Vijver MJ, et al. Systematic analysis of breast cancer morphology uncovers stromal features associated with survival. Sci Transl Med. 2011;3(108):108ra13.
20. Veta M, van Diest PJ, Willems SM, Wang H, Madab-

hushi A, Cruz-Roa A, et al. Assessment of algorithms for mitosis detection in breast cancer histopathology images. Med Image Anal. 2015;20(1):237-48.
21. Couture HD, Williams LA, Geradts J, Nyante SJ, Butler EN, Marron JS, et al. Image analysis with deep learning to predict breast cancer grade, ER status, histologic subtype, and intrinsic subtype. NPJ Breast Cancer. 2018;4:30.
22. Ibrahim A, Gamble P, Jaroensri R, Abdelsamea MM, Mermel CH, Chen PC, et al. Artificial intelligence in digital breast pathology: Techniques and applications. Breast. 2020;49:267-73.
23. Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thürlimann B, Senn HJ. Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol. 2009;20(8):1319-29.
24. Vijayashree R, Aruthra P, Rao KR. A comparison of manual and automated methods of quantitation of oestrogen/progesterone receptor expression in breast carcinoma. J Clin Diagn Res. 2015;9(3):Ec01-5.
25. Saha M, Arun I, Ahmed R, Chatterjee S, Chakraborty C. HscoreNet: A Deep network for estrogen and progesterone scoring using breast IHC images. Pattern Recognition. 2020;102:107200.
26. Khameneh FD, Razavi S, Kamasak M. Automated segmentation of cell membranes to evaluate HER2 status in whole slide images using a modified deep learning network. Comput Biol Med. 2019;110:164-74.
27. Tewary S, Mukhopadhyay S. HER2 Molecular Marker Scoring Using Transfer Learning and Decision Level Fusion. J Digit Imaging. 2021;34(3):667-77.
28. Vandenberghe ME, Scott MLJ, Scorer PW, Söderberg M, Balcerzak D, Barker C. Relevance of deep learning to facilitate the diagnosis of HER2 status in breast cancer. Scientific Reports. 2017;7(1):45938.
29. Tewary S, Arun I, Ahmed R, Chatterjee S, Mukhopadhyay S. AutoIHC-Analyzer: computer-assisted microscopy for automated membrane extraction/scoring in HER2 molecular markers. Journal of Microscopy. 2020;281.
30. Yue M, Zhang J, Wang X, Yan K, Cai L, Tian K, et al. Can AI-assisted microscope facilitate breast HER2 interpretation? A multi-institutional ring study. Virchows Arch. 2021;479(3):443-9.
31. Chaurasia A, Culurciello E, editors. Linknet: Exploiting encoder representations for efficient semantic segmentation. 2017 IEEE Visual Communications and Image Processing (VCIP); 2017: IEEE.
32. Wang JL, Ruan J, He SM, Wu CC, Ye GL, Zhou JF, et al. Detection of Her2 Scores and Magnification from Whole Slide Images in Multi -task Convolutional Network. 2018 11th International Symposium on Computational Intelligence and Design. International Symposium on Computational Intelligence and Design2018. p. 7-10.
33. Joseph J, Roudier MP, Narayanan PL, Augulis R, Ros VR, Pritchard A, et al. Proliferation Tumour Marker Network (PTM-NET) for the identification of tumour region in Ki67 stained breast cancer whole slide images. Sci Rep. 2019;9(1):12845.
34. Feng M, Deng Y, Yang L, Jing Q, Zhang Z, Xu L, et al. Automated quantitative analysis of Ki-67 staining and HE images recognition and registration based on whole tissue sections in breast carcinoma. Diagnostic Pathology. 2020;15(1):65.
35. Swiderska-Chadaj Z, Gallego J, Gonzalez-Lopez L, Bueno G. Detection of Ki67 Hot-Spots of Invasive Breast Cancer Based on Convolutional Neural Networks Applied to Mutual Information of H&E and Ki67 Whole Slide Images. Applied Sciences [Internet]. 2020; 10(21).
36. Geread RS, Sivanandarajah A, Brouwer ER, Wood GA, Androutsos D, Faragalla H, et al. piNET-An Automated Proliferation Index Calculator Framework for Ki67 Breast Cancer Images. Cancers (Basel). 2020;13(1).
37. Cai L, Yan K, Bu H, Yue M, Dong P, Wang X, et al. Improving Ki67 assessment concordance by the use of an artificial intelligence-empowered microscope: a multi-institutional ring study. Histopathology. 2021;79(4):544-55.
38. Negahbani F, Sabzi R, Pakniyat Jahromi B, Firouzabadi D, Movahedi F, Kohandel Shirazi M, et al. PathoNet introduced as a deep neural network backend for eval-

uation of Ki-67 and tumor-infiltrating lymphocytes in breast cancer. Scientific Reports. 2021;11(1):8489.
39. Fulawka L, Blaszczyk J, Tabakov M, Halon A. Assessment of Ki-67 proliferation index with deep learning in DCIS (ductal carcinoma in situ). Scientific Reports. 2022;12(1):3166.
40. Saha M, Chakraborty C, Arun I, Ahmed R, Chatterjee S. An Advanced Deep Learning Approach for Ki-67 Stained Hotspot Detection and Proliferation Rate Scoring for Prognostic Evaluation of Breast Cancer. Scientific Reports. 2017;7(1):3213.
41. Zhu J, Liu M, Li X. Progress on deep learning in digital pathology of breast cancer: a narrative review. Gland Surg. 2022;11(4):751-66.
42. Beck AH, Sangoi AR, Leung S, Marinelli RJ, Nielsen TO, Van De Vijver MJ, et al. Systematic analysis of breast cancer morphology uncovers stromal features associated with survival. Science translational medicine. 2011;3(108):108ra13-ra13.
43. https://digitalpathologyassociation.org/.
44. The Cancer Imaging Archive. (n.d.). Retrieved from https://www.cancerimagingarchive.net/.
45. Coulter C, McKay F, Hallowell N, Browning L, Colling R, Macklin P, et al. Understanding the ethical and legal considerations of Digital Pathology. The Journal of Pathology: Clinical Research. 2021;8.
46. Sorell T, Rajpoot N, Verrill C. Ethical issues in computational pathology. J Med Ethics. 2022;48(4):278-84.
47. Gurcan MN, Boucheron LE, Can A, Madabhushi A, Rajpoot NM, Yener B. Histopathological image analysis: a review. IEEE Rev Biomed Eng. 2009;2:147-71.
48. Vandenberghe ME, Scott ML, Scorer PW, Söderberg M, Balcerzak D, Barker C. Relevance of deep learning to facilitate the diagnosis of HER2 status in breast cancer. Scientific reports. 2017;7(1):1-11.
49. Yuan Y. Modelling the spatial heterogeneity and molecular correlates of lymphocytic infiltration in triple-negative breast cancer. Journal of The Royal Society Interface. 2015;12(103):20141153.
50. Tőkés T, Tőkés AM, Szentmártoni G, Kiszner G, Madaras L, Kulka J, et al. Expression of cell cycle markers is predictive of the response to primary systemic therapy of locally advanced breast cancer. Virchows Arch. 2016;468(6):675-86.
51. Lu C, Xu H, Xu J, Gilmore H, Mandal M, Madabhushi A. Multi-Pass Adaptive Voting for Nuclei Detection in Histopathological Images. Sci Rep. 2016;6:33985.
52. Mungle T, Tewary S, Arun I, Basak B, Agarwal S, Ahmed R, et al. Automated characterization and counting of Ki-67 protein for breast cancer prognosis: A quantitative immunohistochemistry approach. Comput Methods Programs Biomed. 2017;139:149-61.
53. La Barbera L, Larson AN, Rawlinson J, Aubin C-E. In silico patient-specific optimization of correction strategies for thoracic adolescent idiopathic scoliosis. Clinical Biomechanics. 2021;81:105200.
54. Singh R, Chubb L, Pantanowitz L, Parwani A. Standardization in digital pathology: Supplement 145 of the DICOM standards. Journal of pathology informatics. 2011;2(1):23.