Review paper

The role of computational pathology in predicting expression of selected immunohistochemical markers

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Immunohistochemistry (IHC) remains a cornerstone of precision oncology, providing essential diagnostic, prognostic, and predictive molecular data. However, the traditional manual assessment of IHC slides faces persistent challenges regarding interobserver variability, reproducibility, and high diagnostic workloads. To address these limitations, artificial intelligence (AI) is increasingly integrated into computational pathology workflows. This paper outlines the current landscape of AI-assisted IHC, highlighting significant trends such as automated scoring, explainable AI, virtual staining, and multiplex analysis. We explore the transition from basic image analysis to advanced deep learning architectures capable of predicting specific IHC biomarker expression directly from standard hematoxylin and eosin morphology. Furthermore, we examine the commercial ecosystem of these tools and highlight the critical pre-analytical bottlenecks that hinder widespread clinical adoption. Finally, we present a practical case study demonstrating an automated, deep learning-based pipeline for quantifying CD34-positive myeloblasts in acute myeloid leukemia and myelodysplastic syndromes. Ultimately, while AI holds vast potential to optimize turnaround times and streamline laboratory triage, its successful clinical implementation will depend on seamless integration into existing digital pathology platforms and standardization of pre-analytical variables, moving the field from fragmented algorithms to reliable, standard-of-care diagnostic tools.

Key words: digital pathology, computational pathology, stain conversion.

Introduction

The principles of immunohistochemistry (IHC) were first practically demonstrated in 1941 when Coons *et al.* [1] utilized fluorescein-labelled antibodies to localize pneumococcal antigens. Since then, the methodology has rapidly evolved through the integration of robust enzymatic markers and the landmark discovery of monoclonal antibodies, transforming into an essential multidisciplinary tool in modern pathology [2].

Despite the advent of molecular genetics, IHC remains a cornerstone of routine histopathology. The method relies primarily on high-affinity interactions between specific antibodies and target antigens within tissue sections [3, 4]. This targeted biochemical reaction enables the direct visualization of protein expression and cellular localization, providing critical diagnostic, prognostic, and predictive information that dictates patient management across a wide spectrum of diseases.

However, the rapidly evolving field of precision oncology increasingly demands highly detailed and objective data [5]. The traditional manual assessment of IHC slides, whether qualitative or quantitative, faces

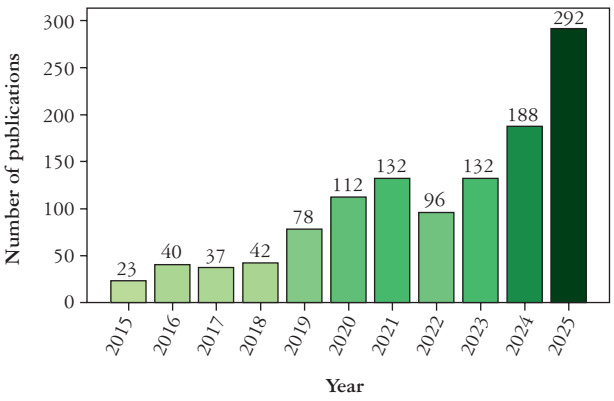

Figure 1. The increasing number of AI/deep learning in immunohistochemistry publications in PubMed (17.03.2026)

well-documented challenges related to inherent interobserver variability and scoring inconsistencies [6]. To address these subjective limitations, artificial intelligence (AI) has been progressively integrated into pathological workflows [7–9]. Evolving from basic image analysis to advanced deep learning architectures, AI has emerged as a computational tool designed to standardize evaluations, handle complex tissue data and ultimately augment the diagnostic capabilities of modern pathologists [10].

Recent advances in AI-assisted immunohistochemistry

Over recent years, there has been a significant surge in the application of AI within the field of IHC (Figure 1). By developing advanced AI algorithms, researchers and clinicians aim to overcome the inherent limitations of manual slide interpretation. Currently, four predominant trends define the landscape of AI-driven IHC: AI-assisted scoring, Explainable AI, AI-assisted (virtual) staining, and Multiplex IHC analysis.

AI-assisted scoring

The quantitative assessment of IHC slides has traditionally been hindered by substantial inter- and intra-observer variability [11]. Artificial intelligence-assisted scoring systems address this challenge by providing standardized, high-throughput analysis [12]. These tools improve diagnostic consistency by generating metrics for a marker expression, such as Ki-67 proliferation indices [13–15] or human epidermal growth factor receptor 2 (HER2) status [16] ensuring highly reproducible results. In the context of breast cancer, AI-assisted scoring algorithms for estrogen receptor (ER) and progesterone receptor (PR) have demonstrated high levels of agreement with manual interpretations by focusing on the proportion of positive tumour cells. The implementation of these AI tools as a "second-reader" significantly enhances interobserver concordance, particularly in cases of low-positive expression where manual agreement is typically lower. For example, AI assistance has been shown to increase pathologist agreement 93.0–96.5% for ER and 84.6–91.5% for PR [17]. In the specific context of folate receptor α in ovarian cancer, such technologies are becoming essential because interpretation is inherently semi-quantitative and susceptible to interobserver variability. While pathologists demonstrate high reproducibility in estimating percentage positivity (intraclass correlation coefficient = 0.89), discrepancies are primarily confined to borderline cases involving 65–85% positivity and tumours with intermediate staining near clinical decision thresholds [18]. Deep learning-based systems can accurately segment tumour areas and quantify membranous staining, while rule-based tools allow for customizable algorithmic thresholding to calculate the percentage of tumour cells with 2+/3+ expression. The integration of these AI-assisted tools for scoring supports more standardized therapeutic decision-making and helps mitigate dilemmas by capturing more nuance in expression patterns [18].

Explainable AI

As deep learning models increase in complexity, interpretability remains a critical barrier to clinical adoption. A considerable number of these algorithms function as "black boxes," meaning their internal decision-making processes are unclear to the user [19]. Explainable AI focuses on demystifying neural networks to make their outputs clinically transparent [20]. By generating visual aids such as heatmaps and attention maps, Explainable AI identifies the specific morphological features or cellular regions that influenced a particular diagnostic prediction and highlights it so this transparency allows pathologists to validate, if the algorithm is focusing on relevant biological signals.

AI-assisted staining

Artificial intelligence-assisted staining, often referred to as "virtual staining", utilizes computational models such as Generative Adversarial Networks to transform non-stained [21] or standard hematoxylin and eosin (HE) stained tissue sections into virtual IHC representations [22]. By using data from autofluorescence (AF) imaging, HE-stained and IHC-stained slides in combination with deep learning, researchers developed an algorithm that demonstrated the ability to generate virtual, specific IHC stains based solely on the AF image from completely unlabelled slides [23]. Similar attempts have been made to virtually generate complex breast cancer biomarker panels (HER2, PR, ER, and Ki-67) directly from HE; however, performance in these areas remains constrained by limited data availability [24]. Ultimately,

this technology holds significant potential to reduce reagent costs, accelerate turnaround times, and preserve limited biopsy material.

Multiplex immunohistochemistry

Multiplex IHC allows for the simultaneous detection of multiple biomarkers on a single tissue section, preserving valuable clinical samples and providing a comprehensive view of the tumour microenvironment. The core principle of this process relies on either tagging antibodies with metal isotopes or more frequently, using repetitive cycles of staining to build up multiple layers of stains information on the same slide [25]. Each marker is added one at a time in a step-by-step process. After a specific marker is permanently "locked" onto the tissue, the antibodies are removed, which clears the slide so the next marker can be added without reacting with previous layers. This iterative method allows many different signals to be safely layered onto a single slide, providing a map of cell-to-cell interactions [26–28].

Artificial intelligence is essential for the computational "unmixing" of overlapping signals, a process of digital stain deconvolution that mathematically separates individual biomarker channels from the composite image [27]. By transcending the limitations of human visual perception, AI-based multiplexing enables a faster understanding of cellular infiltration processes and protein co-expression, providing detailed insights into the tumour microenvironment that cannot be quantified using manual methods [28].

Predicting expression of selected immunohistochemical markers

Deep learning models can identify subtle morphological features within the HE tissue architecture that correlate with specific molecular phenotypes. For instance, multiple large-scale studies utilizing diverse architectures, ranging from convolutional neural networks and multiple-instance learning frameworks to vision transformers, have successfully predicted the status of key breast cancer markers, including Ki-67, ER, PR, and HER2. These approaches demonstrate high predictive reliability, frequently achieving area under the curve (AUC) values of 0.85–0.90 across extensive patient cohorts [29–31]. Similarly, in urothelial pathology, models employing clustering-constrained-attention multiple instance learning have predicted HER2 expression in bladder cancer directly from routine HE slides [32].

Other methodologies incorporate generative AI and cross-modality learning. Generative Adversarial Networks have been utilized to map HE tissue patterns to IHC expression, enabling the algorithmic generation of synthetic "virtual stains" for biomarkers such as Ki-67, HER2, PR, and ER [24, 33]. Similar image-to-image translation approaches have been applied to convert HE stains into elastic van Gieson [34], Masson's trichrome staining [35] or periodic acid-Schiff staining [36]. Furthermore, cross-modality frameworks, such as HistoStainAlign, leverage contrastive learning to predict multi-marker expression patterns (including P53, PD-L1, and Ki-67) directly from HE across gastrointestinal and pulmonary tissues [37].

Predicting IHC expression from routine HE morphology offers notable clinical advantages. By extracting predictive molecular insights from universally available HE slides, these algorithms can serve as effective pre-screening or triage tools. However, it is crucial to recognise that the majority of these models remain in the experimental phase. The transition from research-grade algorithms to clinically approved diagnostic tools is a highly complex and time-consuming process that requires extensive multi-centric validation.

Commercial landscape: industry solutions and startup ecosystem in digital pathology

The commercial landscape of digital pathology is currently dominated by solutions targeting the most prevalent solid tumours. Major established companies, such as Roche (uPath), Indica Labs (HALO AP), and Visiopharm, offer dedicated, commercially available image analysis modules for standard IHC markers like HER2, Ki-67, and PD-L1. While several of these algorithms have achieved CE-IVD certification for clinical use in Europe, such as Roche's uPath HER2 and Ki-67 image analysis algorithms, Visiopharm's CE-IVD Breast Panel APPs, and Mindpeak's BreastIHC software, Food and Drug Administration approvals remain relatively scarce and are often strictly validated only for specific scanner and assay combinations. Consequently, a significant portion of these sophisticated IHC analysis tools are distributed strictly as research use only (RUO) products. In laboratory practice, the primary value of these software packages lies in standardizing quantitative tasks, reducing turnaround times, and minimizing inter-observer variability in breast or lung cancer.

Beyond standard cell counting, innovative digital pathology companies are commercializing advanced predictive analytics as RUO software suites and biopharma service platforms. A prominent clinically approved example is Owkin's MSIntuit CRC, a diagnostic product that predicts microsatellite instability (MSI), a phenotype traditionally evaluated *via* physical mismatch repair IHC staining, directly from routine HE slides. This product functions as an automated pre-screening filter, significantly reducing the volume and costs of confirmatory physical testing. Concurrently, companies like PathAI (with their AI-based measurement of PD-L1 product) and Paige (offering the Paige Breast HER2 module) market AI-powered

software designed to objectively score complex physical IHC markers or to extract predictive phenotypic data for clinical trial stratification. Furthermore, early commercial products offering "virtual staining", the algorithmic generation of synthetic IHC representations from HE inputs, exemplified by the AI-powered virtual staining platforms from companies like PictorLabs, are being introduced to biopharmaceutical partners to preserve limited biopsy tissue and reduce reagent costs.

However, major bottlenecks hinder the widespread adoption of these predictive products in routine hospital settings. These tools are highly sensitive to pre-analytical variables, such as laboratory-specific tissue fixation times, staining protocols, and scanner colour profiles.

Challenges in automated immunohistochemistry assessment

The implementation of AI in pathology represents a significant step toward improving objective reproducibility. The primary challenge of automated IHC assessment is the detection of cancer cells in which the expression of IHC markers is evaluated. During this process, AI-algorithms encounter numerous confounding factors that can affect accurate assessment, such as the expression of IHC markers in healthy tissues.

An example is the quantification of CD34-positive myeloblast cells in hematopathology. While CD34 is a primary indicator for myeloblast cell percentage, it is also constitutively expressed by the vascular endothelium (Figure 2).

A pathologist can contextually disregard these vascular signals based on morphological structure, but automated systems often struggle to distinguish endothelial structures from cancer cell profiles without specialized "context-aware" training. This lack of insight can lead to a significant overestimation by incorporating physiological co-expression or non-specific binding into the final result.

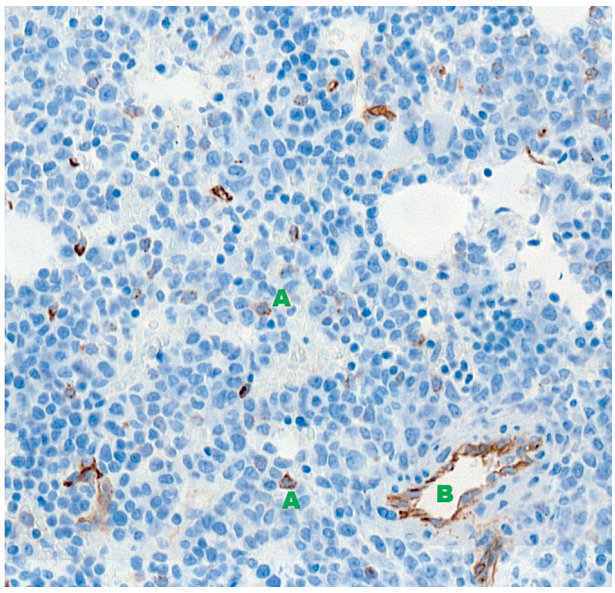

Figure 2. Example of CD34 stained trepanobiopsy, where A – CD34 marker absorbed by myeloblast cells, B – CD34 marker absorbed by the vascular endothelium

Significant bottlenecks remain in this area due to the inherent sensitivity of tissue samples to confounding factors introduced during the pre-analytical stage. The reliability of automated IHC assessment is fundamentally tethered to slide quality, as several key variables can compromise algorithmic accuracy and lead to the misinterpretation of biological signals [38].

These challenges begin with physical pre-scanning artifacts, such as tissue folds, tears, dust or precipitates on the slide, or the presence of margin pigments and inks. Unlike specialists who can contextually disregard and filter these defects, standard deep learning models may struggle to classify these regions properly, often leading to false positives or segmentation errors. To mitigate this, the field has moved toward automated quality control tools, which identify and mask these artifacts before the analysis phase to ensure data integrity [39].

In addition to surface-level defects, there are also biochemical changes, such as prolonged cold ischemia – the period between surgical excision and tissue fixation – which trigger a cascade of enzymatic degradation. In the context of automated IHC, this causes a heterogeneous loss of immunogenicity that AI models may incorrectly interpret as low biological expression rather than technical degradation, potentially leading to the under-diagnosis of therapeutic targets [40]. Similarly, the kinetics of formaldehyde-induced cross-linking are critical for stabilizing target proteins. Deviations in this process, such as under-fixation in high-volume specimens, result in a "raw" necrotic core and staining patterns confined exclusively to the edges that distort the quantitative landscape. Furthermore, over-fixation masks epitopes, requiring aggressive antigen retrieval that can further damage tissue morphology and skew algorithmic results [41].

In specialized cases like bone marrow trepanobiopsy, the decalcification process introduces significant variability. While EDTA-based chelation maintains higher antigenic fidelity, aggressive acid-based methods can irreversibly denature protein structures and lead to the "halo effect" – an osmotic artifact occurring at the bone-stroma interface, often leads to uneven staining of margins, which hinders the precise automated segmentation of tumour cells in these critical areas [42].

Case study: automated CD34 myeloblast grading in acute myeloid leukemia and myelodysplastic syndromes

Accurate assessment of CD34-positive myeloblasts is a key step in the diagnostic grading of myelodys-

plastic syndromes (MDS) and acute myeloid leukemia (AML) [43]. The process requires careful visual inspection of regions across the slide, and can be time-consuming and subject to inter- and intra-observer variability [44–46]. To address these challenges, we developed an automated computational framework designed to estimate blast percentage directly from whole-slide images (WSI) of CD34-stained bone marrow biopsies. The overall workflow of the proposed method is illustrated in Figure 3.

The proposed approach is based on automated identification and counting of individual cells within histopathology images and aggregates this information to assess the overall grade of the WSI.

Cell detection and vascular exclusion

The analysis begins with preprocessing of the digital WSI to identify tissue regions and exclude non-informative areas such as background and slide borders. The remaining tissue regions are then divided into smaller image patches that can be processed individually by the algorithm. Within these patches, vascular structures are identified and excluded from further processing. In CD34-stained bone marrow biopsies, endothelial cells lining blood vessels are also strongly CD34-positive and could therefore be misinterpreted if not removed [47, 48]. To avoid this potential source of false-positive detections, vascular structures are first localized using a detector trained with the You Only Look Once architecture (YOLO11n) [49]. The detected vessels are then precisely segmented using the Segment Anything Model (SAM3) [50], and the resulting masks are applied to remove these regions from further analysis.

After this preprocessing step, the algorithm performs automated detection of individual hematopoietic cells. A deep learning-based object detection model, trained using the YOLOv12s architecture [51, 52], is used to localize cells and simultaneously classify them into two categories: CD34-positive cells and CD34-negative cells. Next, for each analysed patch, the algorithm computes the ratio of CD34-positive cells and CD34-negative cells. To align with clinically relevant diagnostic categories, the estimated blast percentage is mapped into commonly used grading thresholds ($< 5\%$, $\geq 5 < 10\%$, $\geq 10 < 20\%$, and $\geq 20\%$) [53, 54], which corresponds to established criteria used in the classification of MDS and AML.

Slide-level aggregation

Because a single WSI contains thousands of patches, the algorithm combines information from all analysed patches to generate a final slide-level prediction. The system aggregates the spatial extent of regions with different predicted blast proportions, assigning greater importance to areas with higher pathological burden. This strategy integrates local cellular measurements while preserving the spatial distribution of blasts across the tissue.

Results

The proposed method was evaluated according to two complementary criteria: the detection and quantification of CD34-positive blasts at the region of interest (ROI) level, and the assignment of a final grade at the WSI level.

At the ROI level, the automated quantification framework produced results consistent with expert annotations, with differences in estimated blast percentage typically remaining within a small margin. After discretising these results into four ordinal grades ($< 5\%$, $\geq 5 < 10\%$, $\geq 10 < 20\%$, and $\geq 20\%$), Cohen's κ reached $= 0.760$, indicating substantial agreement [55].

At the WSI level, the method was evaluated on an independent cohort of 231 WSI. Patch-level predictions were aggregated into slide-level results using the proposed aggregation strategy, yielding an AUC of 0.75. The system identified all pathological cases (100% sensitivity), ensuring that no clinically relevant cases were missed. At the same time, approximately 37% of negative slides were automatically excluded from further diagnostic review. Consequently, automated pre-screening could reduce the workload associated with routine slide evaluation while maintaining diagnostic safety. Overall, this case study demonstrates how explicit cell-level quantification of CD34-positive

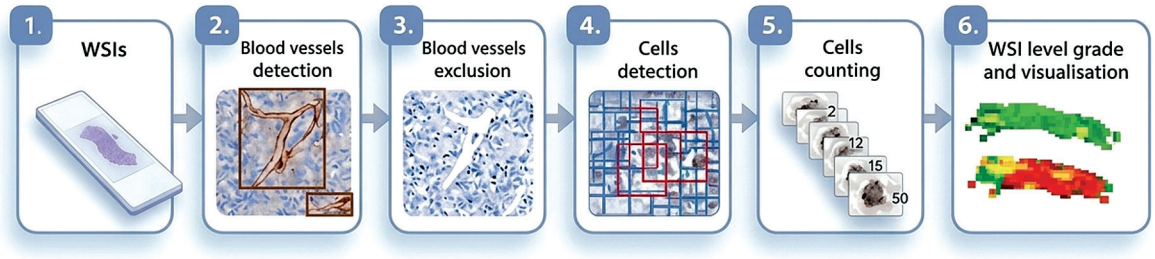

Figure 3. Overview of the proposed pipeline for automated CD34-positive blast quantification, where WSI – whole-slide images

blasts can provide a reliable foundation for AI-assisted grading of AML and MDS in digital pathology.

Discussion

The expanding body of literature underscores the transformative impact of AI on IHC. As demonstrated, AI applications are reshaping the diagnostic landscape, ranging from automated scoring systems that expedite pathologist workflows and triage urgent cases, to advanced predictive models and virtual staining technologies designed to minimize turnaround times and reduce reagent costs.

While the long-term potential of these computational tools is vast, the immediate future will be defined by their practical clinical implementation. The field must transition from fragmented, standalone experimental algorithms to seamless, universally compatible integration within existing Laboratory Information Systems and overarching digital pathology platforms.

Conclusions

Consequently, rather than a sudden diagnostic revolution, pathology is entering an era of pragmatic, gradual integration. By addressing these critical bottlenecks and overcoming the algorithmic "black-box" trust deficit, AI can move beyond the research phase to become an indispensable, standard-of-care tool in modern precision oncology, augmenting rather than replacing the pathologist's expertise.

Disclosures

1. Institutional review board statement: Not applicable.
2. Assistance with the article: We gratefully acknowledge Polish high-performance computing infrastructure PLGrid (HPC Center: ACK Cyfronet AGH) for providing computer facilities and support within computational grant no. PLG/2025/018531.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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