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Review Article

Digital and Computational Pathology Applications in Bladder Cancer: Novel Tools Addressing Clinically Pressing Needs

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ABSTRACT

Bladder cancer (BC) remains a major disease burden in terms of incidence, morbidity, mortality, and economic cost. Deciphering the intrinsic molecular subtypes and identification of key drivers of BC has yielded successful novel therapeutic strategies. Advances in computational and digital pathology are reshaping the field of anatomical pathology. This review offers an update on the most relevant computational algorithms in digital pathology that have been proposed to enhance BC management. These tools promise to enhance diagnostics, staging, and grading accuracy and streamline efficiency while advancing practice consistency. Computational applications that enable intrinsic molecular classification, predict response to neoadjuvant therapy, and identify targets of therapy are also reviewed.

Introduction

The Promise of Digital Pathology

Digital pathology (DP), powered by advances in computational sciences, is poised to revolutionize cancer management (Fig. 1). In addition to being non–tissue destructive, integration of DP in routine clinical care has numerous advantages for precise, efficient health care delivery, research innovations, education of future generations of trainees, and more globally, in reducing health care costs.[1,2] Although there are concerns that artificial intelligence (AI) may potentiate health care disparities due to different accessibility,[3] it can help identify and minimize such disparities by generating unbiased algorithms that work evenly well across ethnic groups.[4,5] A major impediment in accurate personalized cancer management is the subjectivity of diagnosis. AI-based diagnoses introduce objective and reproducible criteria, reducing inter- and intra-observer variability, thus providing data for the development of accurate predictive and prognostic models. As the cost of image storage reduces, routine digitization of all patient slides will increase efficiency of histology workflow and reduce personnel cost, as shown in a financial projection for DP implementation, resulting in increased productivity in the pathology lab for both pathologists and histotechnologists, resulting in full-time equivalent capacity gains and operational cost savings.[2] Access to all previous histopathology slides for review and comparison during the extended follow-up needed

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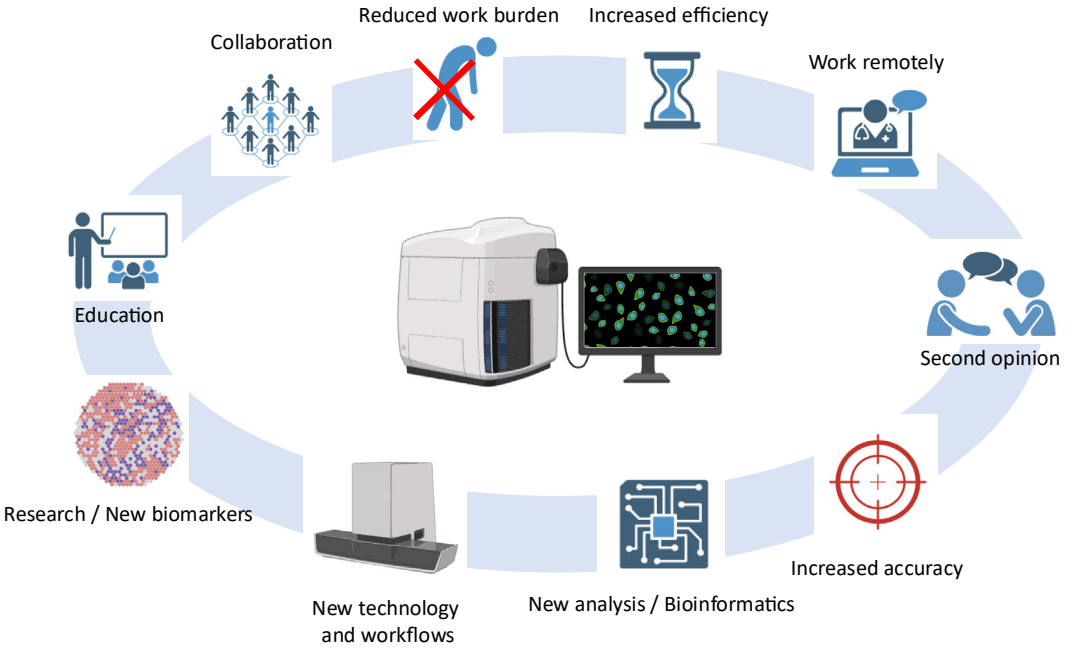

Figure 1.
Advantages and potentials of establishing digital pathology and artificial intelligence (AI) solutions. AI brings the promise of reducing the burden of the working pathologist by addressing tasks that are cumbersome, repetitive, and prone to errors. It could also bring comfort to the pathologist, allowing for modification of workstations, working remotely from home and easily consulting colleagues, asking for external opinions on cases, and fostering collaborations. It is an important tool for teaching pathologists in training. Also, AI allows for quantification and development of new biomarkers, pushing research forward, and facilitating technological advances, bridging the world of big data analysis. This figure was created using BioRender.

for management of BC will improve accurate tracking of patients' progress.[6] Furthermore, digitization facilitates expert consultation on difficult cases, enabling underserved and underdeveloped regions to triage patients appropriately, thus reducing health care disparities.

A major impediment for computational scientists is access to large, well-annotated data sets. Large, well-annotated training and test sets are needed in contemporary research, which requires the analysis of innumerable complex data points. Routine digitization of all patient slides will enable the development of image databases from clinical cases diagnosed by experts. This will facilitate the development of multiinstitutional cohorts. Application of AI-based analytical tools will permit the objective analysis and quantification of innumerable clinically significant data points.[7,8] Such a large volume of information cannot otherwise be discerned or collated into a clinically useful document using optical microscopes and currently available reporting formats. The power of computational pathology (cPATH) enables the collection and analysis of data not only from cancer cells but also from the surrounding stroma and tumor microenvironment. This captures the unique cancer-host dynamic, which is an important determinant of patient outcomes. The exponential increase in computational abilities combined with the availability of large digital data sets promises a future where "multiomic" models, capable of collating clinically relevant information from pathology, radiology, and other management modalities, will inform future clinical care.

Epidemiology and Current Management of Bladder Cancer

Bladder cancer (BC) represents the second most common urologic malignancy after prostate cancer[9] and overall, the ninth most common cancer. The Globocan 2020 report found age-standardized incidence and mortality rates of 5.6 and 1.9 per 100,000 worldwide, respectively. It is estimated that the worldwide incidence and mortality will increase by 62.5% and 73.7% from 2022 to 2040, respectively.[10] BC continues to be one of the most expensive malignancies to treat, primarily due to the lack of accurate predictive nomograms. Precise diagnostic and management tools are therefore needed to manage this disease efficiently and cost-effectively.

Grade and stage are major determinants of the clinical management of BC. The treatment strategies are quite different for patients with or without muscularis propria (MP) invasion. Majority of non−muscle-invasive BC (NMIBC) are characterized by local recurrences and treated with intravesical therapy. A subset of NMIBC (~10%-15%) progresses to muscle-invasive BCs (MIBCs). The lack of accurate tools to distinguish progressors from non-progressors necessitates similar follow-up protocols for all patients. This leads to frequent invasive and expensive cystoscopic examinations. The need for frequent hospital follow-up visits leads to reduced patient compliance and additional economic burdens on health care systems and patients.[11-13] MIBC have a universally poor prognosis and are treated aggressively with a combination of neoadjuvant chemotherapy (NAC) and cystectomy. Invasion of MP (detrusor muscle) is the only unifying feature of the otherwise biologically diverse MIBC. The 2020 consensus molecular classification divides MIBC into 4 categories, each with its unique targets and response to current treatment regimens.[14-18] Translating these molecular profiles into daily practice, however, has been challenging.[19-23] Most patients therefore continue to be treated with a combination of NAC and radical resection. AI algorithms capable of predicting molecular subtypes hold promise for better distinctions between subtypes of MIBC and can therefore drive individualized management.[24-26]

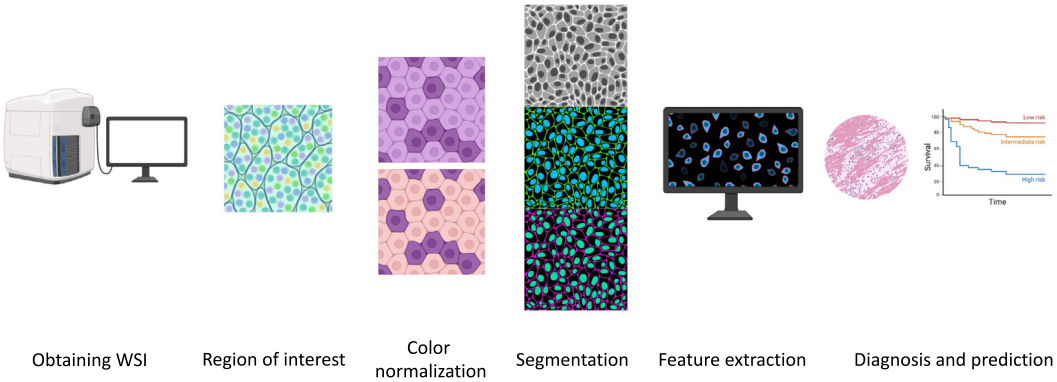

Figure 2.
Illustration of strategies and solutions used in digital image analysis for improving diagnosis and prediction. Development of AI models from whole slide images (WSI) often start by defining regions of interest and are followed by sequential steps to assure normalization of analysis. Segmentation is useful for concentrating on specific aspects and extracting them for generating predictions, ultimately creating a model for diagnosis or prognostication of patients. Adapted from a previous article.[38] This figure was created using BioRender.

Voided urine and bladder washes contain exfoliated cells, protein material, and immune cells. They are rich sources of biologic information that can be used to develop noninvasive tools for early diagnosis and for posttreatment follow-up. Computational models capable of integrating DP characteristics from transurethral resection (TUR), urine cytology, clinical features, radiologic features, and molecular data[27-30] hold great promise for the precise, effective, and efficient management of BC.[31-37] An ongoing clinical trial (NCT05825950) is testing the feasibility of integrating of cPATH into the management of BC. If successful, this will be an important milestone toward the integration of AI into patient care.

Despite the accelerated and promising developments of AI, the complexities of analyzing and interpreting BC on whole slide images (WSI) pose several challenges. In this review, we systematically discuss the advances, remaining gaps in knowledge, and limitations in the application of AI algorithms for the early noninvasive diagnosis and follow-up of NMIBC and MIBC (Fig. 2; Tables 1 and 2).

Digital Pathology Tools for Tissue and Cell Segmentation

Attempts to apply image analysis in BC started in 2004 when Glotsos et al[40] demonstrated the feasibility of accurate nuclear segmentation of urothelial cells. Accurate segmentation of urothelial cells from surrounding tissue and differentiation of benign urothelial proliferation from preneoplastic and neoplastic processes was the initial focus of digital evaluation.[48,51,58] In 2014, Al-Janabi et al[43] demonstrated the feasibility of diagnosing urinary tract pathology on WSI. In this study, adjudication of 13 discrepancies from the 100 cases analyzed showed WSI analysis to be of greater accuracy in 6, whereas 5 showed major discrepancies with important prognostic implications. In the remaining, 2 cases both light microscopy and WSI analyses were considered to give an imperfect description. Inflammatory conditions and posttreatment changes may both induce epithelial changes that mimic flat urothelial carcinoma in situ or produce papillary architecture mimicking papillary urothelial carcinoma (PUC) on both urine cytology and TUR specimens.[66] Both over- and underdiagnosis reduce the precision of predictive and prognostic models, thereby hindering effective treatment. Chen et al[53] developed an AI algorithm that accurately diagnosed BC, with an area under the curve (AUC) of 0.96, 0.89, and 0.94 in the training, test, and validation cohorts, respectively. Importantly, they demonstrated that BC can be discriminated against cystitis with an AUC of 0.93. This is an important step forward in reducing over- and underdiagnosis of BC, as well as in the development of ancillary tools to facilitate AI-aided clinical workflow.

Niazi et al[51] provided a multiclass image segmentation model validated on pT1 (lamina propria invasive BC) TUR specimens. Their model accurately segregated the urothelium from other components of the bladder wall such as the lamina propria, muscularis mucosa (MM), and propria, and regions of hemorrhage, inflammation, and cautery artifact. Limitations of the method identified by pathologists included failure to identify glandular cells, sclerotic stroma within muscle, and necrotic foci within lamina propria. A similar strategy was developed by Wetteland et al,[48] who presented a model using tiles extracted at 3 different magnifications, 25×, 100×, and 400×, to segment out tumor background, damaged tissue, muscle, stroma, blood, and urothelium in PUC. Such algorithms can be used as screening tools to direct a pathologist's attention toward diagnostically relevant areas. They are also an important basis for pursuing further automated analyses (such as prediction of grade or invasion based on features of specific portions of tissue).[58] A limitation of the study, however, was imprecision of the model on lower scale magnifications and a small sample size, meaning that expansion of these investigations is needed.

The ability of content-based image retrieval systems to extract similar cases diagnosed previously could aid pathologists in decision making and increase diagnostic accuracy. Kalra and colleagues trained an algorithm with WSIs from 32 tumor types other than BC to retrieve similar cancer-type images achieving 96% accuracy.[67] In another published study, Khosravi et al[68] used WSI from bladder, breast, or lung cancer as a general label to classify cancer types, achieving 100% accuracy.

More recently, Jansen et al[46] showed that 3D segmentation and reconstruction from histologic images is possible, allowing better evaluation of the spatial relationship between BC and MP. This forward-looking method, if validated, can help refine the assessment of patterns of invasion and staging.

Improvements in DP tools for tissue and cell segmentation allow for the development of advanced neural networks capable of aiding the automated diagnosis of cancer. These first steps therefore are an important landmark in advances in DP for the diagnosis of BC.

Table 1
Summary of publications including digital pathology solutions in BC for diagnostic, grading, staging and prognostic purposes

Context	Aim	Cohort size	Findings	Methodology	Author
Grading	Comparing grading on glass slides vs image analysis	Cohorts 1 and 2 (197/100)	Image analysis correlated well with pathologist's grading and predicted survival	Digitalized Feulgen-stained slides and operator-selected regions of interest Image analysis strategies (object, texture, graph)	Choi et al[39]
Overall analysis	Providing a method for automated segmentation of nuclei of urothelial cells	50 BC biopsies	94% of nuclei were correctly delineated	Support vector machine clustering	Glotsos et al[40]
Grading	Assessing NMIBC grade	129 NMIBC patients	Both methodologies resulted in high accuracy in grading (85.3% and 83.7%)	Support vector machines and probabilistic neural networks	Spyridonos et al[41]
Clinical outcome	Predicting disease recurrence by analyzing nuclear features	127 superficial BC	Prediction rates of 87.95% and 91.41% for nonrecurrent and recurrent cases	Feedforward neural networks	Tasoulis et al[42]
Diagnosis	Establishing the feasibility of diagnosing urinary tract pathology	100 consecutive urinary tract biopsies (kidney and urothelial pathology)	Concordance of 87% with previous glass-slide diagnosis	WSI	Al-Janabi et al[43]
Grading	Predicting slide-level WHO2004 grade	913 WSI of BC (102 low-grade papillary urothelial carcinoma; 811 high-grade papillary urothelial carcinoma) 620/193/100 training/validation/ test cohorts	The system (AUC 0.97) matches the performance of 17 board-certified pathologists System descriptions of BC features	Segmentation Three neural networks (s-net, d-net, a-net)	Zhang et al[44]
Clinical outcome	Identifying tumor budding	WSI of 100 MIBC patients	Digital analysis of tumor budding was the most significant discriminator (HR = 2.59) of DSS	Quantitative image analysis based on detection of nuclei and panCK positive cells (Definiens Tissue Phenomics) Segmentation CNN	Brieu et al[45]
Overall analysis	Providing a 3D reconstruction from histologic images	16 cases, 12 BC and 4 benign (26-30 sections per case)	Full 3D visualization and evaluation of spatial relationship of BC and muscularis propria	3D segmentation	Jansen et al[46]
Invasion	Discrimination of Ta and T1 BC	1177 BC (460 pTa, 717 pT1)	Accuracy of 91-96% (supervised learning method) Accuracy of 84% (automated CNN model) Desmoplasia was the most important feature	Pipelines for detection of desmoplasia, retraction and eosinophilic cytoplasm	Yin et al[47]
Diagnosis and Overall analysis	Providing a system for segmentation of urothelium, damaged tissue, muscle, stroma, blood and background	32/7 cross-validation/inference cohorts (papillary urothelial carcinoma)	Multiscale models (TRI-model) perform better than MONO- and DI-models F1-score of 96.5% (97.6% for urothelium class alone)	TRI-model (multiscale model) VGG16 network	Wetteland et al[48]
Grading	Predicting slide-level WHO2004 grade	328 TUR of 232 patients (NMIBC)	Moderate agreement with the pathologist's grading (k = 0.48) Sensitivity/Specificity/Accuracy 0.71/0.76/0.74	U-Net based segmentation Two individual single-scale neural networks	Jansen et al[49]

Grading	Discriminating low grade (PUNLMP and low-grade tumors) and high grade (high grade and invasive) tumors	607 WSI (425/182 training/test cohorts) 378 cases (TCGA validation cohort)	F1-score of 0.91 and 0.99 High risk patients with poorer OS (HR of digital pathology analysis 1.958; of WHO/ISUP grade 1.945)	Bladder4Net CNN	Barrios et al[50]
Diagnosis and overall analysis	Providing a method for multiclass image segmentation	54/53 WSI of T1 bladder biopsies (primary/secondary datasets)	Segmentation accuracy of 8.93 and 8.87 out of 10 Accurate identification of bladder layers and minimization of time to annotate slides	Segmentation U-Net	Niazi et al[51]
Clinical outcome	Predicting positive lymph nodes	307 MIBC patients (294 TCGA, 13 own cohort) TCGA 146/73/75 training/validation/testing cohorts	A combined model with AI risk score outperformed clinicopathological features alone (AUC 0.807)	Spatially resolved prediction maps and lymphocyte infiltration	Harmon et al[52]
Diagnosis and clinical outcome	Using machine learning for diagnosing and predicting prognosis of BC	643 WSI (108 radical/partial cystectomies, 406 TCGA) divided in training/test/validation cohorts Also included: 53 normal bladder and 39 cystitis FFPE cases and 37 normal bladder TCGA cases	Diagnosis: AUC 0.96, 0.89 and 0.94 Distinction from cystitis: AUC 0.93 Risk stratification: independent prognostic factor concerning OS The integrated nomogram performed better than clinicopathological parameters alone (AUC 0.777, 0.838 and 0.813 for 1-, 3- and 5-y OS)	Image segmentation and datamining (CellProfiler) Machine learning algorithm Integration with clinical information	Chen et al[53]
Overall analysis	Using AE1/AE3-stained WSI for detecting different patterns of disease	136 WSI of MIBC	Final accuracy of 0.90 in a multiclass scenario, surpassing other clustering-based methods	Unsupervised deep learning	García et al[54]
Clinical outcome	Predicting NMIBC recurrence	125 NMIBC (TUR) patients (95/30 training/test cohorts)	The model predicts 2-y recurrence after TUR with 90% (support vector machine) and 86.7% (random forest) probability	Nuclear extraction process Nuclear morphologic and texture (CellProfiler) Machine learning: support vector machine and random forest algorithms	Tokuyama et al[55]
Clinical outcome	Predicting response to neoadjuvant chemotherapy in MIBC	122 TMA of MIBC, preneoadjuvant chemotherapy (66/56 cohorts)	Accuracy 65-73% Resistance-favored factors: diverse cell morphology, stromal burden, cell orientation entropy, low CD8/FoxP3 ratio	QuPath TMA dearrayer Segmentation and IHC Image texture and nuclear morphometrics	Mi et al[56]
Grading	Comparing grading on glass slides vs on digital screen	48 cases of TUR/biopsies (noninvasive papillary urothelial carcinomas) PUNLMP excluded	Cohen kappa score (agreement) of 0.78 (WHO2004 grade) and 0.82 (WHO1973) Noninferiority to pathologist's grade	Scan at 40× objective	Colling et al[57]
Grading	Providing a tissue segmentation map, a WHO2004 heatmap and a predicted slide-level WHO2004 grade	300 WSI of TUR (NMIBC) 220/30/50 training/validation/testing cohorts	Sensitivity/specificity/accuracy 0.85/1.00/0.90	TRI_{grade} Storage in "pyramid format" Segmentation CNN	Wetteland et al[58]

(*continued on next page*)

Table 1 (*continued*)

Context	Aim	Cohort size	Findings	Methodology	Author
Diagnosis and clinical outcome	Diagnosing BC and predicting OS of MIBC	926 WSI (887 tumor, 39 normal) of 412 MIBC patients (TCGA) + 250 WSI (150 tumor, 100 normal) of 150 MIBC patients (own cohort)	Diagnostic model (BCMIL) with accuracy 0.987 (junior pathologist 0.876, expert uropathologists 0.991-0.993) Prognostic model (MibcMLP) was an independent predictor of OS (HR = 2.414, multivariable) Features predicting high mortality (atypia and vascular spaces); low mortality (immune cells)	Segmentation Heatmap of attention scores Weakly supervised neural network	Zheng et al[38]
Clinical outcome	Predicting 1 y and 5 y RFS	359/281 patients (pTa/pT1) with minimum 1/5 y follow-up	The combined model performed better (AUC 0.62/0.76 for 1/5 y RFS) than a multivariable logistic model with clinicopathological features alone (AUC 0.58/0.57 for 1/5 y RFS)	U-Net based segmentation GRU classification network	Lucas et al[59]
Grading	Discriminating low and high-grade NMIBC	20 cases of TUR (NMIBC)	Accuracy 0.90 (0.91 for high grade and 0.89 for low grade)	Deep learning CNN Visualization/map (Grad-CAM)	Mundhada et al[60]
Grading and invasion	Discriminating HGMI, HGNMI and LGNMI BC	854 WSI (TUR) from 692 patients Training set and 3 validation sets (313/95/201/83)	AUC 0.878, 0.870 and 0.874 in the validation sets Noninferiority to pathologist's grading (higher than junior pathologist, similar to intermediate pathologist, lower than senior pathologist) Identification of muscle invasion with AUC 0.850, specificity 0.941, sensitivity 0.743	Segmentation ScanNet (CNN)	Pan et al[61]
Grading	Discriminating low and high-grade noninvasive papillary urothelial carcinoma	371 patients	Accuracy of 88%, AUC of 0.94	Segmentation Morphometric analysis	Slotman et al[62]

3D, three-dimensional; AUC, area under the curve; BC, bladder cancer; CK, cytokeratin; CNN, convolutional neural network; DSS, disease-specific survival; FFPE, formalin-fixed paraffin-embedded; GRU, gated recurrent unit; LGNMI, low-grade nonmuscle invasive; HGMI, high-grade muscle-invasive; HGNMI, high-grade nonmuscle invasive; HR, hazard ratio; MIBC, muscle-invasive bladder cancer; NMIBC, nonmuscle invasive bladder cancer; OS, overall survival; PUNLMP, papillary urothelial neoplasm of low malignant potential; RFS, relapse-free survival; TCGA, The Cancer Genome Atlas; TMA, tissue microarray; TUR, transurethral resection; WHO, World Health Organization; WSI, whole slide image.

Table 2
Summary of publications including digital pathology solutions in BC for predicting molecular subtypes

Context	Aim	Cohort size	Findings	Methodology	Author
Predicting molecular subtype	Predicting molecular subtype of MIBC	407 MIBC (TCGA) and 16 MIBC (own cohort)	The model performed as well as or better (AUC 0.85-0.89) than standard pathologist's analysis Providing pathologist's with variables identified in the model increased overall accuracy by 20%	CNN CAM	Woerl et al[24]
Predicting molecular subtype	Predicting TMB	253 BC (TCGA)	Accuracy of 73% (AUC 0.75 in discriminating high/low TMB) Impact on OS	Segmentation and tumor detection Transfer learning Deep convolutional networks	Xu et al[63]
Predicting molecular subtype	Prediction of TIL infiltration and *FGFR3* mutational status	418 BC (TCGA)	TIL percentage and *FGFR3* are inversely correlated AUC of TIL percentage in identifying *FGFR3* mutations 0.76, superior to pathologist's TIL scoring (AUC 0.71)	CNN for identifying TILs TIL percentage for predicting FGFR3 mutations	Velmahos et al[64]
Predicting molecular subtype	Predicting FGFR3 mutational status	327 MIBC (TCGA) and 182 cases (own cohort; 121 MIBC, 34 pT1, 27 pTa)	AUC of 0.70-0.73 for predicting FGFR3 mutant cases, outperforming pathologist's assessment	Shufflenet with 2 output neurons	Loeffler et al[25]
Predicting molecular subtype	Establishing a combined radiological and pathologic model for discriminating luminal and basal molecular subtypes	127 patients with BC	The combined radiological and pathologic features achieved better performance (AUC 0.89) than a unimodal approach	CellProfiler CNN	Wu et al[65]

AUC, area under the curve; BC, bladder cancer; CAM, class activation maps; CNN, convolutional neural network; MIBC, muscle-invasive bladder cancer; NMIBC, nonmuscle invasive bladder cancer; OS, overall survival; TCGA, The Cancer Genome Atlas; TIL, tumor infiltrating lymphocyte; TMB, tumor mutational burden.

Digital Pathology Applications for Urine Analysis

Urine accumulates shedding tumor cells and metabolites from the renal pelvis, ureter, and bladder. It therefore offers a unique opportunity for extracting morphologic, molecular, and metabolic information for BC. Urine analysis can therefore be leveraged as a cost-effective tool for early diagnosis, for noninvasive posttreatment surveillance, and for the identification of predictive and prognostic markers.

BC is inherently immunogenic, and clinical specimens from urine cytology may contain extensive inflammation and debris making identification of neoplastic cells challenging. The ability of AI algorithms to accurately differentiate neoplastic from nonneoplastic cells[69] can be leveraged to reduce false-negative as well as false-positive calls. Additionally, AI algorithms can help develop masks that remove diagnostically irrelevant areas (floaters, hemorrhage, and other debris), thereby aiding a more focused and efficient evaluation of urine samples by pathologists.

In a recent study of 2405 scanned ThinPrep slides of urine cytology (both voided and instrumented urine), a deep learning algorithm identified high-grade BC with a sensitivity and specificity of 79.5% and 84.5%, respectively, reaching an AUC of 0.88.[70] A multicenter trial utilized VisioCyt, a deep learning-based automated image processing tool, and demonstrated that it outperformed human reads using the Paris classification, with a sensitivity of 84.9% vs 43%, respectively.[71] The validation phase of this study showed an overall diagnostic sensitivity of 80.9% and specificity of 61.8% in urine samples of 391 patients. The sensitivity increased to 93.7% for high-grade tumors. VisioCyt also achieved a 66.7% sensitivity for low-grade tumors, which is quite remarkable, considering these tumors are not reliably identified by routine cytologic examination.[72] This is important since the overall interobserver agreement (kappa value) for classifying urine samples using the Paris system (the current clinical classification for categorizing urine samples regarding risk of BC) is reported to be 0.32-0.36,[73,74] with most disagreements occurring within the indeterminate categories.[75] This is clinically important, given that the result of the cytology human assessment will determine the clinical action, ranging from follow-up to an invasive procedure (TUR or resection, depending on the clinical context).

Urine metabolites have been studied as potential biomarkers for the early diagnosis and posttreatment follow-up of BC. Shao et al[76] profiled 87 BC and 65 controls to identify inidazole acetic acid as a biomarker for BC. Using an AI model (decision tree), they achieved an accuracy of 76.6% with a sensitivity and specificity of 71.0 and 86.0%, respectively. Early vs late-stage BC demonstrated unique metabolites that can be used to develop algorithms to predict progression. Kouznetsova et al[77] developed a model that predicted metabolite class with 82% accuracy with an AUC of 0.84 on a training data set.

cPATH models that combine morphologic analyses with metabolic characteristics and relevant clinical data points will aid in the development of reliable noninvasive tools for the early diagnosis and follow-up of patients with BC. Use of urine samples in routine clinical workup will thus increase patient comfort and compliance, reduce cost, and improve the overall outcomes of BC.

Digital Pathology Applications for Bladder Cancer Grading

Grading is a relevant component of histologic assessment of BC that informs intravesical therapy for NMIBC. The subjectivity of dichotomizing a continuum of molecular morphologic

changes into the current 2-tiered 2022 World Health Organization (WHO) classification of PUC is challenging and introduces extensive inter- and intraobserver differences in diagnosis. Despite the introduction of a cutoff of >5% high-grade papillary urothelial carcinoma component to classify a tumor as being high grade,[78,79] intratumoral grade heterogeneity, and the architectural distortion and cauterization artifacts encountered in TUR specimens, make the accurate categorization difficult.[78,80-82]

As early as 1997, Choi et al[39] explored image analysis (object, texture, and graph) as a means of grading BC in digitalized Feulgen-stained slides. Several studies have since assessed the utility of DP solutions for BC grading and have sought to improve interobserver variability.[41,57,61,62] Colling et al[57] compared grading on glass slides vs WSI. They found digital grading to be noninferior for both, the WHO 2004 and the WHO 1973 grading schemes ($k = 0.78$ and $k = 0.82$, respectively). Pan et al[61] also showed noninferiority of their AI algorithm to pathologists' grading. The model outperformed 2 junior pathologists, matched the performance level of 2 intermediate-level pathologists, but was inferior to one of the 2 senior pathologists. Limitations of the study included being single-centered, a time-consuming process for full annotation (which could be solved by partial annotation and weak supervision), but mostly the fact that it was not designed to identify specifically carcinoma in situ, which has important clinical implications.

One of the largest studies using AI in BC grading was conducted by Zhang et al.[44] Their deep learning model used 3 neural networks and achieved an AUC of 0.97 for discriminating high- and low-grade PUC, matching the average performance of a panel of 17 board-certified pathologists. The algorithm incorporated tumor region segmentation and feature-aware attention maps to detect several features such as loss of polarity, pleomorphism, mitotic index, prominence of nucleoli, crowding, etc. Several studies have used publicly available digital image repositories (eg, The Cancer Genome Atlas) to validate the performance of their AI algorithms. Barrios et al[50] used Bladder4Net to discriminate low-grade tumors (including papillary urothelial neoplasms of low malignant potential) from high-grade tumors, using their own cohort and The Cancer Genome Atlas as a validation cohort. The model achieved a F1 score (harmonic mean of precision and recall) of 0.91-0.99. The algorithm developed by Wetteland et al[58] achieved an accuracy of 0.90 in discriminating WHO 2004 grade of NMIBC on TUR specimens, same as the performance demonstrated by Mundhada et al[60] for discriminating low- and high-grade NMIBC in TUR specimens. The work of Wetteland et al[58] was, however, limited by having no noncancerous material. The authors also raise the question that these algorithms are designed and trained in high-quality hematoxylin and eosin slides, homogeneous in staining, and it remains unknown how they would operate on lower quality or more variable hematoxylin and eosin preparations. More recently, Slotman et al[62] described combined nuclear morphometry and automated mitotic figure counts to differentiate grades of noninvasive PUC.

Additional studies have achieved "good" levels of performance with AI algorithms predicting slide-level grades. Jansen et al[49] showed moderate agreement with the pathologist's grading of NMIBC on TUR ($k = 0.48$) and an accuracy of 0.74. However, the algorithm assessed tumor grade on a case level based on the majority vote of the classifications of patches, assuming a homogeneous tumor, a weakness that does not address the frequent grade heterogeneity in a given tumor.[83]

Integrating AI grading algorithms into clinical diagnosis will enable the identification of a novel grading system that truly reflects the biology of a patient's cancer and accurately predicts time to recurrence and progression. Defining these essential quantitative elements of grading has the potential to drastically improve pathologic assessment and provide a starting point from which to improve the overall outcome of BC.

The gold standard against which current AI models are tested, however, remains a human read, which themselves are subject to inherent variability. Furthermore, most of the studies use the best representative image rather than all slides and levels to develop the grading model. This approach does not fully capture the tumor heterogeneity so common in BC. Finally, the differences in cohorts and varying inclusion and exclusion criteria make it difficult to perform meta-analyses to gain insight into the progress and applicability of the various models.

Digital Pathology Applications for Bladder Cancer Staging

In NMIBC, identifying and assessing the extent of lamina propria invasion is relevant to staging (pT1 vs pTa/pTis) and prognostication. pT1 tumors with extensive lamina propria invasion have worse outcomes compared with those with limited lamina propria invasion[84-87] However, there is a lack of consensus on how to best measure and quantify the depth of lamina propria invasion.[84] Several approaches have been proposed, including those using histologic landmarks (eg, MM or vascular plexus) and those using a micrometric approach. To date, none of these systems have found widespread acceptance due to difficulty in assessment in the clinical setting.[84,88]

The identification of true MP invasion is an important determinant of considering radical cystectomy. However, the identification of MP invasion is often challenging due to the lack of orientation of transurethral resection of bladder tumor (TURBT) fragments or the presence of cautery artifacts and masking inflammation. In addition, the obliteration of identifiable muscle bundles by extensively invasive tumor and/or myofibroblastic reaction and repair also makes the distinction difficult. The normally thin, discontinuous, and wispy MM can, at times, become hyperplastic, adding another diagnostic challenge. All these findings introduce variability in pathologists' interpretations. Ultimately, overcalling of MP invasion results in substantial morbidity from radical cystectomy, whereas underdiagnosis results in delayed treatment.

A study by Yin et al[47] discriminated pTa and pT1 BC using 2 different AI algorithms designed to detect features of invasion, such as desmoplasia, retraction artifact, and eosinophilic cytoplasm associated with paradoxical differentiation in small clusters of invasive cells. The supervised learning method with feature extraction was superior (accuracy of 0.91%-0.96%) to the completely automated model based on convolutional neural networks (accuracy of 84%). An analysis of all the features used in the study identified desmoplasia as the most significant distinguishing feature. This study, however, excluded morphologically challenging cases, a subset that usually requires the most support for an accurate diagnosis.

Pan et al[61] developed an AI algorithm for identifying MP invasion that achieved an AUC of 0.85 (0.94 specificity and 0.74 sensitivity). Zheng et al[38] have developed a deep learning weakly supervised diagnostic model for MIBC (the BlcaMIL) with a diagnostic accuracy of 0.99. Their model surpassed the performance of a junior pathologist (0.88) and was comparable with one of 2 expert uropathologists (0.991-0.993) in the external validation cohort. Limitations identified by the authors include

factors already discriminated in other AI investigations, including need for larger sample sizes, use of single-centered retrospective data, and variations in slide preparation in different centers (which can be minored with color normalization approaches, after careful validation).

There has been substantial progress in the development of AI models capable of accurately diagnosing and stratifying NMIBC. In order for AI to be integrated into clinical care, the next logical step would include developing the capability to analyze and collate findings from all clinical slides, rather than on a representative level. Capturing tumor heterogeneity will inform precise treatment strategies that will have a significant impact on outcomes and health care costs.

Computational Pathology for Prognostic and Predictive Models

AI can discern, collate, and analyze many more morphologic features than the human eye. Computational models can stratify these innumerable morphologic features for their relevance and combine them with clinical features to devise accurate predictive and prognostic algorithms.[89]

Computational AI assessment of nuclear features to predict disease recurrence in NMIBC has been attempted as early as 2006.[42] Tokuyama et al[55] developed an AI algorithm that detected nuclear features predicting 2-year recurrence after TURBT with 86.7%-90% accuracy. Lucas et al[59] proposed a combined model (deep learning algorithm of pathology features integrated with clinical data) that outperformed clinical features alone in predicting relapse-free survival at 1 and 5 years of follow-up. Use of clinicomorphologic features of primary TURBT to predict recurrence and progression of disease is much needed to reduce frequent invasive procedures like cystoscopic examinations and TUR.

Many studies have developed algorithms to predict response to treatment and outcomes of MIBC. Different histologic patterns of MIBC are associated with varying aggressiveness and prognosis.[49,90] Jimenez et al[91] described 3 distinct invasive growth patterns that predict recurrence. Using immunohistochemical stained WSI, García et al[54] developed a method to classify normal, infiltrative, nodular, and trabecular histologic patterns. Combined morphologic and molecular features of MIBC can be leveraged to devise a MIBC-specific AI-based grading system that can improve treatment strategies and prognosis.

Tumor budding assessment, increasingly recommended for assessment of colorectal cancer, reflects tumor epithelial-to-mesenchymal transition, an important determinant of cancer outcomes.[92] This is a time-consuming task in the routine of a pathologist. Molecular alterations in the epithelial-to-mesenchymal transition pathway play a role in MIBC with sarcomatoid urothelial carcinoma subtype representing its clearest manifestation.[93] AI assessment of tumor budding, defined as the presence of up to 4 cancer cells at the invasive front of a tumor, when combined with certain clinical features can stratify MIBC into disease-specific survival groups.[45] Using immunofluorescence labeling of individual tumor cells with pancytokeratins, the image analysis-based tumor budding detection model of Brieu et al[45] was the most significant predictor of disease-specific survival. Although the best definition and approach to quantifying tumor budding (eg, number, density, and invasive front vs intratumoral budding) are still under discussion in BC, the above study reinforces the potential clinical relevance of utilizing computational models in predicting outcomes.[94]

Harmon et al[52] used an AI model including the assessment of lymphocyte infiltration for predicting lymph node metastases in BC. When added to established parameters, the model outperformed (AUC of 0.81) standard clinicopathological features (pT stage, lymphovascular invasion, and age). If validated in additional studies, inclusion of such models in clinical workflow can help urologists determine the extent of lymph node dissection at radical cystectomy.

To date, clinical tools for predicting response to NAC are limited, resulting in some patients not taking the most benefit from this therapy, which has associated toxicity, leading to significant morbidity. Mi et al[56] developed an algorithm focused on texture, nuclear morphometric features, and select immune markers to predict response to NAC in MIBC. The algorithm identified factors associated with resistance to chemotherapy such as diverse cell morphology, increased stromal burden, cell orientation entropy, and low CD8/FoxP3 ratio (denoting an immune-suppressive microenvironment). It achieved an accuracy of 0.65-0.73 in predicting benefits from NAC. Other studies have also demonstrated the utility of AI in predicting outcomes in MIBC.[38,53] Accurate prediction of response to treatment would allow for the exploration of alternate/targeted treatment upfront for patients predicted to be chemoresistant. Such nuanced treatment regimens will improve disease outcomes, which is much needed in MIBC and BC in general.

Almost 50% of MIBC develop lymph node metastasis. The metastases are often small, and in high-volume centers, lymph node screening becomes time-consuming. Wu et al[65] developed an AI model using WSI to look for lymph node micrometastases that are otherwise hard to spot. Their algorithm performed better than both experienced and junior pathologists. Integrating such tools has the potential to increase efficiency and reduce errors in diagnosis.

Digital Pathology and Predicting Molecular Subtype, Molecular Targets of Therapy, and Immunotherapy Response

Gene expression-based molecular subtypes of MIBC can inform clinical management. However, it is currently impractical to perform genome-wide molecular testing on all BC patients on tissue samples, due to constraints in time and cost. Therefore, there is a strong interest in identifying markers on routine histopathology that can serve as surrogates for the molecular subtypes.[95,96] Studies have attempted the prediction of molecular subtypes using immunohistochemical expression of markers such as GATA3, FOXA1, and CK5/6 with conflicting results,[19-23] likely due to the inherent spatial heterogeneity of BC.[97] To date, no easy-to-implement and quick panel of biomarkers has been introduced in the clinic to determine the full molecular subtypes and therefore advise on the most appropriate therapy. The study by Woerl et al[24] applied deep learning strategies to predict the molecular subtype of MIBC.[95] Their model performed better (AUC of 0.85-0.89) than the pathologist. Interestingly, providing feedback on discriminating morphologic features enhanced the pathologist's accuracy by 20%. A combined model using radiological and pathologic features was able to discriminate luminal and basal cancers with an AUC of 0.89, outperforming unimodal approaches.[98]

Identifying patients for therapies targeting HER2 and FGFR (fibroblast growth factor receptor) pathways is another area where DP could be of benefit. However, again, it is currently impractical to screen all patients for these mutations with targeted molecular assays. Computationally identifying tumors

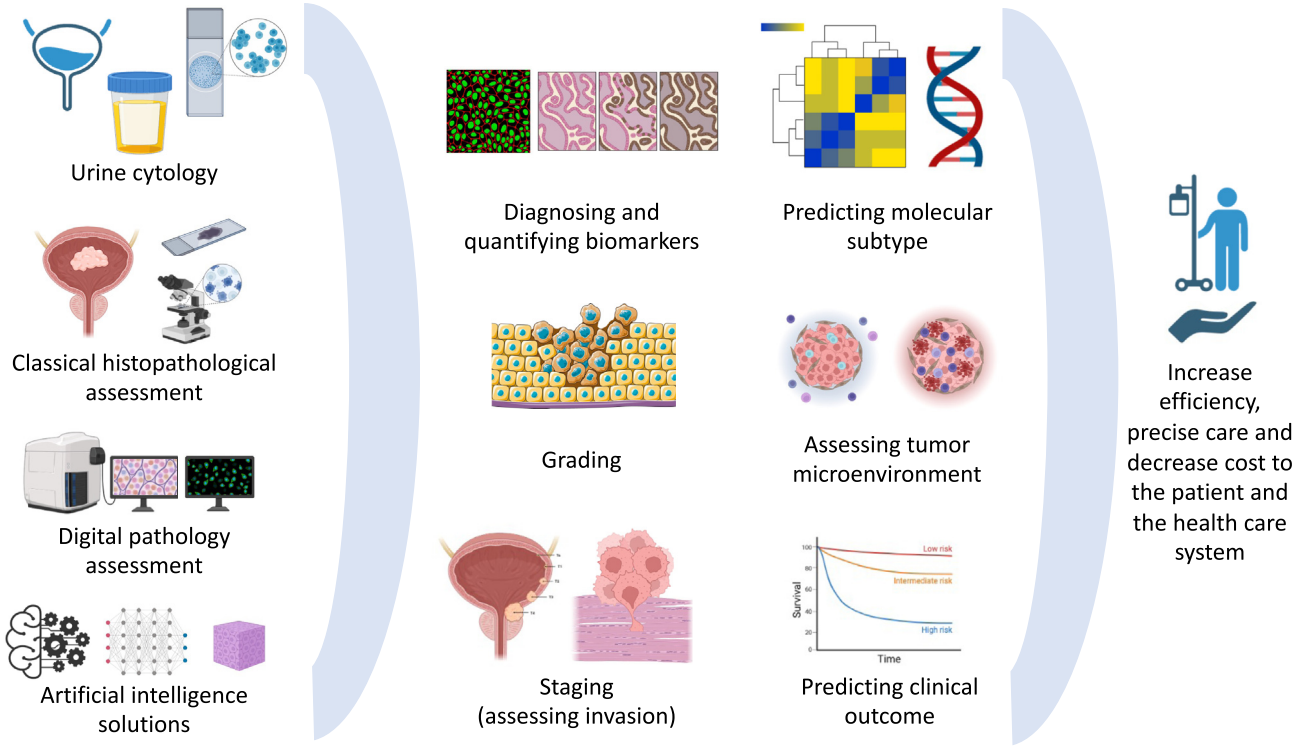

Figure 3.
Integrated approaches for improving bladder cancer patient care. A combined strategy, with both urine cytology and histopathological assessment by experienced pathologists, combined with digital imaging analysis and AI solutions will result in more accurate diagnosis, grading, staging, prediction of molecular subtypes, and response to novel targets of therapy. By allowing for more appropriate risk stratification and selection of treatments, this will ultimately lead to improving bladder cancer patient care. This figure was created using BioRender.

with a higher likelihood of having a *FGFR3* mutation that can be triaged for targeted sequencing constitutes a more cost-effective and efficient strategy.[99] Two studies by Velmahos et al[64] and Loeffler et al[25] developed AI models to predict *FGFR3* mutational status (available in public database as determined by next-generation sequencing), and both outperformed the pathologist predictions based on morphology (AUCs of 0.76 and 0.70-0.73, respectively).

Tumor mutational burden (TMB) is a strong predictor of response to immunotherapy in BC.[100,101] This observation has prompted attempts to use computational approaches to quantitating TMB. Xu et al[63] developed an AI model that discriminates BC with low and high TMB. Their model has an accuracy of 73%, and in identifying these subgroups, it successfully segregated patients with respect to overall survival.

Recently, Xie et al[102] developed an AI-based workflow for the analysis of multiplex immunohistochemistry in BC samples. Using 207 MIBC, the authors showed that a digital image analysis workflow resulted in significant concordance with the individual marker assessment ($P < .001$ for all assessments). Multiplex immunohistochemistry offers many advantages in the assessment and characterization of the tumor microenvironment. It improves the prediction of response to immune checkpoint inhibitors, particularly in limited material. Furthermore, AI-based algorithms may move this technique closer to implementation in clinical practice.

Molecular testing, multiplex immunohistochemistry, and AI tools have made substantial progress toward stratifying MIBC. The best combination of these strategies will enable the development of clinical trials and translation into clinical care.

Challenges of Digital Pathology Implementation

Implementation of a full digital workflow comes with various financial, technical, and logistical challenges related to the changing paradigm of work.[103] All processes have a learning curve. Planning ahead is key for overcoming these challenges and ensuring the ease of implementation. Early engagement of information technology expertise is important to address connectivity, accessibility, system compatibility, and storage space. Rethinking the workflow of pathology labs is required for the optimal introduction of DP. Education and training of staff personnel are needed (eg, avoid placing biopsies close to the edge of the slide).[104]

Challenges related to legal and ethical aspects associated with the introduction of AI are also a concern. Strategies for a responsible adoption of DP and AI systems should be implemented.[3]

Most available studies on quality control report low error scanning rates (below 1.5%[105]), with the need to rescan in 1.84% of the cases.[106] No recognition of the barcode is one of the reported problems. Additional quality control can be introduced with visual assessment by histotechnologist. Attention to preanalytical factors (excess mounting medium, folds, and appropriate placement of coverslips) is important to prevent such issues.

In real-life implementation, concordance in diagnosis using DP is also reported to be high. A previous study achieved 100% major concordance (254/254 reads), with 3 minor discrepancies, having a 98.8% diagnostic concordance rate with glass-slide diagnosis.[106] However, a training and validation phase should be envisioned so that the staff can be familiarized with the digital sign-out.

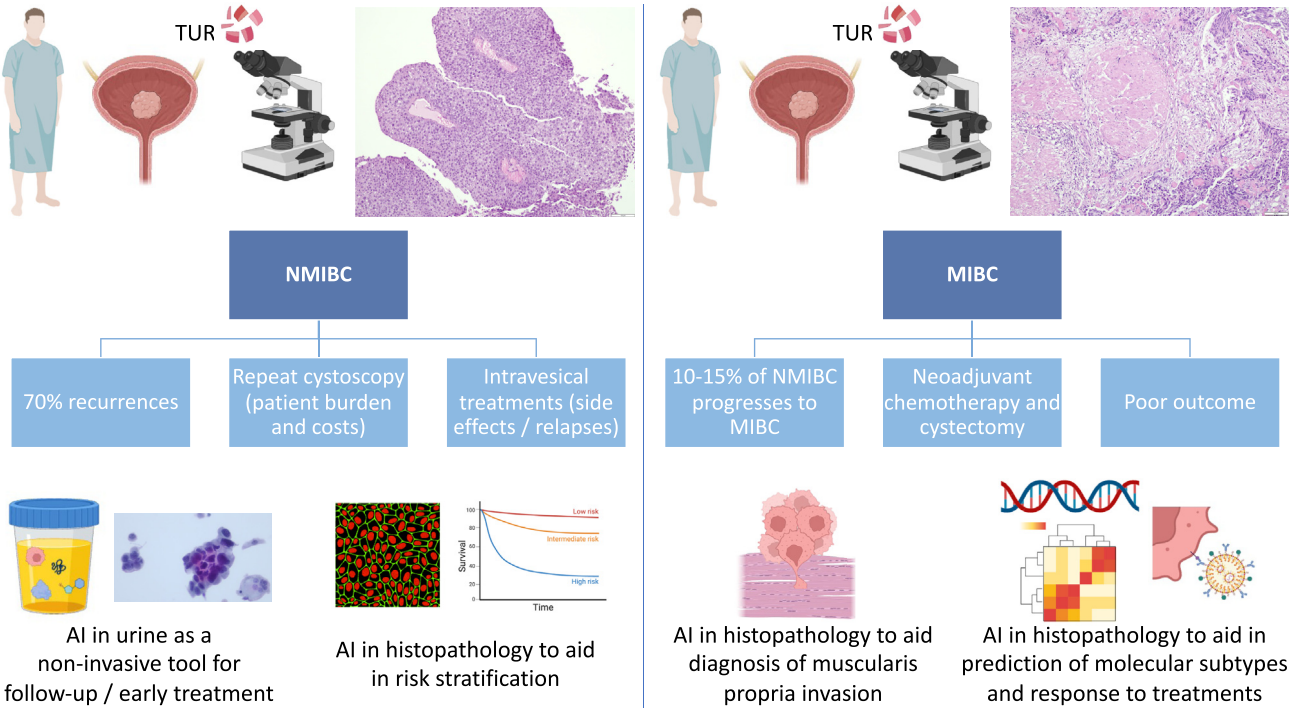

Figure 4.
The bladder cancer patient path, and how AI may improve patient management. On the left side of the panel, the path of a NMIBC patient is shown. The histologic figure shows a high-grade papillary urothelial carcinoma, noninvasive. Patient stratification will determine whether the patient is put on surveillance or receives intravesical treatments. AI in urine analysis may improve the noninvasive monitoring of these patients (a photomicrograph of a urine cytology sample is shown, diagnostic of high-grade urothelial carcinoma as per the Paris classification). Also, AI may highlight features that improve patient risk stratification, allowing for better tailoring of treatment options and follow-up intervals and frequency. On the right side of the panel, the path of a MIBC patient is shown. The histologic figure shows a high-grade papillary urothelial carcinoma that invaded the MP. AI may be of aid in diagnosing invasion of MP, which triggers cystectomy (with/without neoadjuvant chemotherapy). Also, AI may be useful for predicting molecular features and response to targeted therapies. This figure was created using BioRender.

Pathology Reporting in the Digital Era

The workload of pathologists has increased substantially. This is driven by several factors including an increasing population, an aging population with a rising cancer incidence, a multiplication of diagnostic tools and assays requiring interpretation, and an increase in the number of data points to be included in the final diagnosis. Developing a final diagnosis document has therefore become more time-consuming. The longitudinal follow-up of patients with BC also requires pathologists to pull and review slides from previous resections. Furthermore, institutions have progressively limited, if not completely eliminated, administrative and billing support. As a result, formatting documents is often more time-consuming than making a diagnosis. Zhang et al developed algorithms that generate pathology reports and retrieve the correlated images. An image-to-text retrieval method was built to generate diagnostic reports, and a text-to-image retrieval method was built to visualize the image pixels responsible for the reported findings, thereby enhancing the interpretability of the outcomes and substantially increasing accuracy and efficiency. Their model has already achieved 79% accuracy.[107] With the introduction of generative AI, we can anticipate progressive improvement in the performance of similarly designed and trained algorithms. Also, more recently, foundational models for computational pathology such as "Virchow," importing large data sets from several cancers, allowed for cancer recognition with an AUC of 0.95 and show utility for biomarker prediction,[108] which can be very promising for meeting clinical needs in BC.

Conclusions

Advances in computational and DP are changing the clinical and research landscape of anatomical pathology. In this update, we have reviewed AI tools (including deep learning, decision tree, and neural network algorithms) that have been developed and tested to enhance BC diagnostics, grading, and staging accuracy and reliability. Computational applications that enable molecular classification, identify targets for therapy, and predict response to neoadjuvant and immunotherapy are promising. A major limitation of several studies is the use of small cohorts and selection of cases with modest histologic complexity and artifacts. Algorithms trained on carefully selected regions of interest may not perform well in routine clinical cases and may overlook diagnostic information outside of their training program. Studies that use all slides from an unselected cohort will better capture the heterogeneity of BC. This, however, may be challenging as cases containing artifacts from instrumentation, cautery, post-treatment inflammation, and granulomas can compromise a model's reproducibility and accuracy. Nevertheless, this is an important and critical problem to surmount if AI applications are to be included in clinical workflow. The use of large well-annotated carefully phenotyped multiinstitutional cohorts will help account for these challenges and facilitate the development and validation of clinically useful models. The integration of digitized computational platforms into daily practice has the potential to increase efficiency and improve clinical outcomes of patients with BC (Figs. 3 and 4).

Author Contributions

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

Ethics Approval and Consent to Participate

Not applicable.

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