Review Article

The Landscape of Digital Pathology in Transplantation: From the Beginning to the Virtual E-Slide

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Abstract

Background: Digital pathology has progressed over the last two decades, with many clinical and nonclinical applications. Transplantation pathology is a highly specialized field in which the majority of practicing pathologists do not have sufficient expertise to handle critical needs. In this context, digital pathology has proven to be useful as it allows for timely access to expert second-opinion teleconsultation. The aim of this study was to review the experience of the application of digital pathology to the field of transplantation. **Methods:** Papers on this topic were retrieved using PubMed as a search engine. Inclusion criteria were the presence of transplantation setting and the use of any type of digital image with or without the use of image analysis tools; the search was restricted to English language papers published in the 25 years until December 31, 2018. **Results:** Literature regarding digital transplant pathology is mostly about the digital interpretation of posttransplant biopsies (75 vs. 19), with 15/75 (20%) articles focusing on agreement/reproducibility. Several papers concentrated on the correlation between biopsy features assessed by digital image analysis (DIA) and clinical outcome (45/75, 60%). Whole-slide imaging (WSI) only appeared in recent publications, starting from 2011 (13/75, 17.3%). Papers dealing with preimplantation biopsy are less numerous, the majority (13/19, 68.4%) of which focus on diagnostic agreement between digital microscopy and light microscopy (LM), with WSI technology being used in only a small quota of papers (4/19, 21.1%). **Conclusions:** Overall, published studies show good concordance between digital microscopy and LM modalities for diagnosis. DIA has the potential to increase diagnostic reproducibility and facilitate the identification and quantification of histological parameters. Thus, with advancing technology such as faster scanning times, better image resolution, and novel image algorithms, it is likely that WSI will eventually replace LM.

Keywords: Digital pathology, donor biopsy, graft biopsy, image analysis, transplantation

Introduction

Digital pathology has progressed over the last two decades and is being used for several clinical and nonclinical applications. Some of these use cases, including primary diagnosis, second-opinion consultation, archiving, education/training, research, and image analysis. Many studies have been performed on the implementation and validation of digital systems. Several reviews have reported on the concordance between whole-slide imaging (WSI) and conventional light microscopy (LM) in surgical pathology[1,2] and highlighted some of the technical challenges related to WSI in cytology.[3] In addition, several digital image analysis (DIA) tools have been developed over the years, and apart from their role in quantitative image analysis of breast biomarkers, these algorithms have been used mainly for research purposes.

Transplantation pathology is a highly specialized field in which the majority of pathologists do not have enough expertise to handle critical practice needs. Digital pathology can be extremely useful in this regard as it allows general pathologists

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to employ teleconsultation for intraoperative consultation as well as to rapidly gain an expert second opinion. In addition, DIA can be applied to transplant biopsies to facilitate the identification and quantification of several morphological parameters, as well as their spatial relationships.

The aim of this paper was to review the literature on transplantation digital pathology published in the last 25 years and to review the main issues, results, and future directions of the field.

Methods

Papers on this topic were retrieved using PubMed as a search engine. The search was limited to papers written in the English language and published in the 25 years' time span until December 31, 2018, with the following search strategy: "("digital" OR "whole slide imaging" OR "WSI" OR "digital pathology" OR "telepathology" OR "telemedicine" OR "image analysis") AND ("transplant" OR "transplantation" OR "organ" OR "organ procurement" OR "preimplantation biopsy" OR "graft" OR "allograft") AND ("renal" OR "kidney" OR "liver" OR "heart" OR "lung" OR "pancreas")". Inclusion criteria were the presence in the study of the transplantation setting, pre- or post-transplant, and the use of any type of digital pathology image, both with or without the use of image analysis tools. Papers dealing with digital pathology and biopsies but not in transplantation setting, reviews, and commentaries were excluded. Papers retrieved were divided into pre- and post-transplant phase and grouped according to the organ of interest in the study, type of digital pathology, use of image analysis tools, main topic of the study among concordance/reproducibility, assessment of features for organ outcome and rejection, and other morphological or immunohistochemical (IHC) issues.

Distribution of studies

A total of 2207 papers were retrieved with the search strategy, and the main reasons for exclusion on the basis of title and abstract were (i) the absence of the transplantation setting, as the term "transplant" was intended only for tissues in plastic and reconstructive surgery; (ii) the absence of a digitalized image, as the term "digital" was intended for other imaging modalities; and (iii) the use of animal models. The included papers were 93, with the note that a single study[4] comprised both pre- and post-transplant biopsies, so it was counted in both groups. The studies included so represented about 4% of all retrieved items. There were a growing number of publications in the last 15 years as more than 75% of papers have been published after 2004. Subdividing the studies according to the type of digital pathology, it can be seen how the static image modality use has started to decrease after 2008 and how the number of publications using WSI is increasing in the last decade, overcoming the static digitized image in the most recent period 2014–2018. A graphical summary of the distribution of studies over time is shown in Figure 1. Regarding the main issues addressed in the studies, the

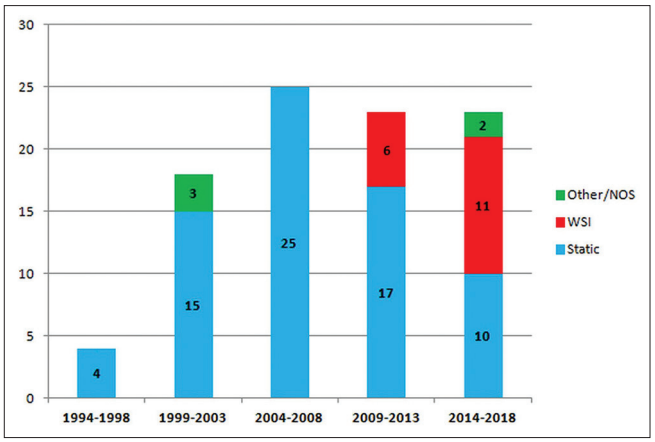

Figure 1: Number of publications over time and according to the type of digital pathology. WSI: Whole-slide imaging, NOS: Not otherwise specified

concordance between modalities was the main topic overall in pretransplant phase papers (14/19, 73.7%), while it was the focus of the study only in 20% (15/75) of posttransplant studies. Indeed, in this group, the correlation of histological features assessed with digital instruments with outcome and the investigation of features related to rejection represented together the most common issues, with total 60% (45/75) of publications. Splitting according to technology type, it can be observed that in studies using WSI, the main topic is the concordance between WSI and conventional LM, both in pretransplant (all 4 studies) and posttransplant (9/13, 69.2%) studies. The assessment of histological features correlated to outcome of organ or with particular attention to rejection was the main topic of the studies using static digitized images (41/70, 58.6%, all posttransplant studies). A diagram of distribution of studies according to transplant phase, type of digital pathology, and main topic is shown in Figure 2.

Modes of digital pathology

Static telepathology requires only a microscope with an attached digital camera connected to a monitor or computer, internet access, and secure sharing software. A remote expert pathologist can view these static images but relies on an on-site pathologist who controls the microscope to capture relevant images that are in focus, which makes this inexpensive system restrictive.[5] This can be overcome with robotic or dynamic telepathology, which allows the remote pathologist to control the microscope using software; however, this robotic system is more expensive, is time-consuming, and demands a high network bandwidth.[5] WSI scanners are essentially a microscope and software-driven robotic stage that methodically moves the slide in the x and y axes under the microscopic lens while simultaneously optimizing the Z-plane focus and photographing each microscopic field.[5] WSI scanners can be tile-based (the most common ones), in which a square photosensor is used to capture multiple tiles adjacent to each other, or line scan-based imaging, in which an oblong photosensor is used to continually capture strips of image data as it sweeps through the slide. The quality of focusing

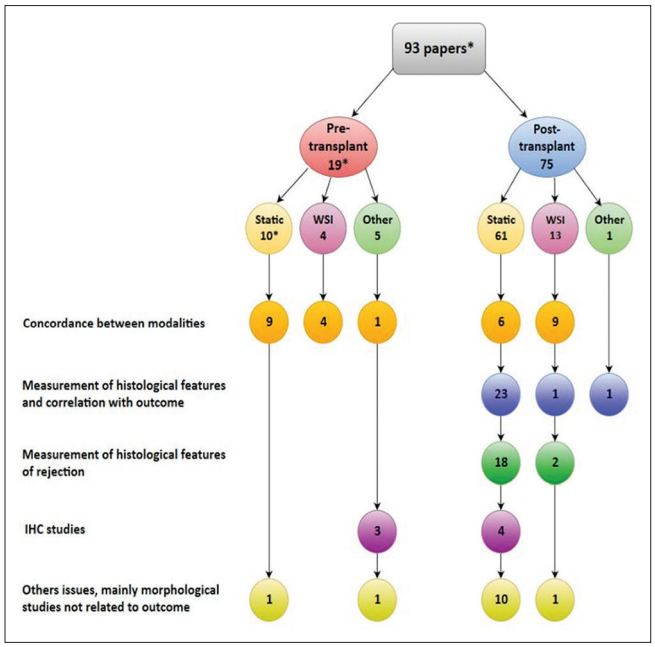

Figure 2: Hierarchy of papers according to transplantation phase, mode of digital pathology, and main topic of study. *A paper is counted in both groups as it comprises both pre- and post-transplant biopsies. IHC: Immunohistochemistry, WSI: Whole-slide imaging

is limited by multiple optical and mechanical parameters, notably the numerical aperture (NA) of the objective and movement resolution on the vertical (*z*) axis. Higher NA allows the distance that can be resolved to become smaller, thus increasing resolution.[6] WSI has proven to be superior in comparison to conventional microscopy in terms of case organization, navigation and annotation of slide, easiness to share for consultation and multiple viewing, and to be reliable for routine surgical pathology diagnosis, after validation of systems.[7] However, scanning time at higher resolutions, storage issues, and costs remain open questions that could have limited widespread adoption of this technology at the beginning; however, nowadays, for academic institutions or community hospitals with a high diagnostic workload, these issues are not to be considered a barrier. Indeed, as reported by a recent international survey, after full implementation of digital pathology, in routine practice, the new step could be the integration of artificial intelligence tools in diagnostic pathology.[8] Finally, hybrid WSI-robotic technology offers pathologists the ability to switch between live robotic viewing and a scanned digital slide.[9] The use of WSI in the transplantation literature only appears after 2011 (13/75, 17.3% of posttransplantation and 4/19, 21.1% of pretransplantation papers).

Telepathology in transplantation

The application of telemedicine to transplantation has lagged significantly compared to other medical fields, despite widespread interest.[10] The clinical benefits of mobile health technologies have been demonstrated in various phases of organ transplantation, including adherence of patients to therapy, clinical monitoring, and increase in life quality of recipients. In addition, in recent years, a number of case series and feasibility studies have highlighted the importance of digital pathology for providing access to expert second opinions. Indeed, this technology can help with real-time allograft selection and assessment of donor/recipient tissue specimens by allowing the teleconsultation of professionals during both pre- and post-transplant phases in medical centers with minimal experience.[10] However, the working scenarios in pre- and post-transplant phases is quite different. The posttransplant phase is best handled by a dedicated subspecialized pathologist, without the need for urgent turnaround times, and if needed availability of ancillary techniques. On the other hand, preimplantation diagnosis can typically be handled by an on-call general pathologist but does need to meet a turnaround time of only a few hours and usually without the luxury of ancillary studies (i.e., diagnoses depend almost entirely on a hematoxylin and eosin stain). In both scenarios, the need for diagnostic teleconsultation may be important.

The vast majority of papers on digital pathology and transplantation published in the last 25 years dealt with the posttransplant biopsy during graft surveillance (75 posttransplant vs. 19 pretransplant articles, 79.8% vs. 20.2%). Minervini *et al*. reported their experience with second-opinion teleconsultation using a static telepathology system between the Mediterranean Institute for Transplantation and Advanced Specialized Therapies in collaboration with the University of Pittsburgh Medical Center.[4] In that study, the authors reviewed 18 posttransplant biopsies and five preimplantation frozen section (FS) liver biopsies. They assessed the agreement rates between the referring and consulting pathologist and the reliability and easiness of telepathology for obtaining a rapid second opinion.[4] Low experience with digital pathology in the pretransplantation phase may be attributed to several reasons. Before the development of contemporary WSI scanners, the acquisition of digital images (e.g., static photographs) required a lengthy amount of time that was inconsistent with the rapid turnaround time needed for preimplantation biopsy assessment. Over time, as imaging devices began to allow dynamic and robotic telemicroscopy, so did the use of telepathology to remotely read intraoperative FSs before organ transplantation.[9]

Digital image analysis in transplantation

Although as stated in recent reviews,[11,12] the risk/benefit ratio and relative value of postimplantation biopsy for graft surveillance could appear to be decreasing, compared to less invasive monitoring techniques, given the development of newer noninvasive imaging and fluid techniques. However, advances in digital imaging techniques, robotics, and computing can provide new "toolkits" enabling pathologists to gain more information from tissue samples and to increase the histopathology value.[11] Indeed, starting from the early 90s, image analysis morphometric studies have been performed mainly for the detection of signs of rejection and prediction

of organ outcome. The absence of time limitation comparing to pretransplant phase allows the pathologist to use ancillary techniques, to digitalize images, and to ask for consultation and perform image analysis, after slide scanning, and take advantage of DIA techniques for precise quantification of morphological features on biopsies. Among the posttransplant studies, 58/75 (77.3%) were carried out using conventional microscopy plus DIA, 8/75 (10.7%) were performed using WSI plus DIA, while 9/75 (12%) did not use DIA techniques. As clarified by Isse *et al*., morphometric software programs, which can range from relatively inexpensive basic macro-driven software for color quantification, too expensive and complex, trainable model-based applications for recognizing and quantifying tissue patterns, now consider WSI.[11] Moreover, the development of multiplex staining DIA algorithms and of deep learning algorithms has been rapidly increasing in recent years, with several applications in cancer pathology, that can be also applicable to transplantation biopsy pathology.[12] Therefore, it is reasonable that in the next two decades, the proportion of WSI versus LM in image analysis studies will be reversed as more image analysis studies will use WSI and deep learning algorithms.

Digital pathology in pre-transplantation

Despite the greater number of published studies on posttransplantation biopsies, there is increasing awareness of the potential to use digital pathology in the pretransplantation phase. Pathologists involved in on-call rotations for the transplant service may be asked to classify lesions found during donor assessment and to evaluate the suitability of organs to transplant from small biopsies. For newly discovered lesions, the pathologist performing these duties needs to define their nature and exclude a malignant neoplasm that would preclude safe transplantation.[13] The studies concerning preimplantation biopsies are summarized in Table 1. Among 19 studies concerning the pretransplant phase, none addressed diagnostic issues of newly discovered lesions. However, given that these lesions are typically examined by means of FS, they are probably incorporated in other more general studies about digital pathology for intraoperative consultation. Most studies on organ assessment (14/19, 73.7%) were mainly about liver and kidney biopsy,[4,14-26] while only a small proportion (5/19, 26.3%)[27-31] dealt with pancreatic islet preparations for transplant. With regard to the type of digital pathology technology used, 12/19 (63.2%) studies discussed DIA applied to LM-acquired images, 4/19 (21.1%) studies used WSI,[15,16,25,26] one study involved only static telepathology without DIA,[4] another referred generally to using a "virtual microscope,"[24] and one did not clarify the type of digital pathology used.[23]

The majority of studies (14/19, 73.7%) concerning the pretransplant phase addressed the agreement/concordance of digital pathology with the conventional LM technique. The assessment of agreement was performed with different statistical tests. Minervini *et al*. reported an agreement rate of 86% between referring pathologist with LM and consultant pathologist with static digital pathology, but they did not specify the agreement rates for the each of the pretransplant cases.[4] Other studies from the same group followed guidelines of the College of American Pathologists for validating WSI systems and compared WSI to LM in the assessment of kidney and liver biopsies. In one of their studies, the intraobserver concordance was excellent ($\kappa = 0.961$). The interobserver concordance was excellent for both LM ($\kappa = 0.903$) and WSI ($\kappa = 0.863$).[26] In another study on the validation of a WSI scanner, the case population included 28 scanned FS slides of the liver and kidney biopsy for organ suitability; the intraobserver concordance was excellent ($\kappa = 0.91$) with an accuracy rate of 86%.[15] Biesterfeld *et al*. analyzed the interobserver concordance in the quantification of macro- and micro-vesicular steatosis in liver biopsies using digital pathology. They found good interobserver agreement ($\kappa > 0.70$) for all degrees of steatosis (correlation coefficient $r > 0.90$ and $r > 0.60$) when the assessment was performed with LM, but the concordance rate was lower when using point grid counting on digitized images. Therefore, they concluded that point grid counting on the digital image does not add value for steatosis quantification.[22] Two other studies analyzed the correlation between macrovesicular steatosis assessed by an experienced pathologist with LM to that assessed by DIA software ($r^2 = 0.426$). One study reported low correlation ($r^2 = 0.426$); however, DIA measurements had stronger correlation with liver function after transplant.[21] In the other study, a high correlation ($r^2 = 0.97$) was found between pathologist's assessment and the DIA method.[18]

Several studies concerned pancreatic islet preparations for islet transplant and compared the assessment of various parameters, including the number of islets, islet equivalents (islets normalized for an average size of 150 μm, IEQ), and purity using different methods. All of them reported high correlation between manual counting on LM[28,29] or on a digitized image[30] and counting using automated/computerized DIA software (determination coefficient $r^2 = 0.91$, $r^2 = 0.78$ and linear coefficient $r > 0.819$, respectively). Three studies compared manual LM and automated DIA software by means of the coefficient of variation (CV), reporting that the CV is lower for automated software compared to manual counting[27,29] and concluding that DIA is reliable for quantification of IEQ and purity.[30] Finally, one study compared three modalities (i.e., manual assessment on LM, manual assessment of digital images, and counting by DIA using software) and reported a high correlation between assessment of digital images and software analysis ($r^2 > 0.8$) and a lower correlation between standard manual assessment and software analysis (r^2 0.62–0.73).[31]

Recently, some authors developed a deep learning model to identify and classify nonsclerosed and sclerosed glomeruli in WSI scans of donor kidney FS biopsies. They reported that their model based on convolutional neural networks yielded results comparable with those achieved by an expert renal pathologist, being robust enough to handle FS artifacts

Table 1: Summary of papers dealing with pre-transplantation phase

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of the study	Results
Minervini *et al*., 2001	Static	102	Various case types, among which 5 donor FS liver biopsies	Consultant telepathology review	Referring pathologist original diagnosis	Agreement rates, descriptive	86% agreement and 14% (only 3% major) disagreement between referring and consultant pathologist
Li *et al*., 2002	LM plus DIA	102	Donor kidney biopsy	DIA software assessment	None	Glomerular volume and sclerosis in different age groups	Glomerular size and global sclerosis increase with age
Benkoel *et al*., 2003	Confocal laser microscopy plus DIA	30	Donor liver biopsy, preimplantation and postreperfusion	DIA assessment of IHC staining for ICAM-1	None	Difference in ICAM-1 expression between preimplantation and postreperfusion biopsies	Higher expression of ICAM-1 in sinusoidal endothelial cells in postreperfusion biopsies
Benkoel *et al*., 2003	Confocal laser microscopy plus DIA	30	Donor liver biopsy, preimplantation and postreperfusion	DIA assessment of IHC staining for F-actin	None	Difference in F-actin expression between preimplantation and postreperfusion biopsies	Significantly lower expression of F-actin in postreperfusion biopsies
Benkoel *et al*., 2003	Confocal laser microscopy plus DIA	30	Donor liver biopsy, preimplantation and postreperfusion	DIA assessment of IHC staining for NaK-ATPase	None	Difference in NaK-ATPase expression between preimplantation and postreperfusion biopsies	Significantly lower expression of NaK-ATPase in postreperfusion biopsies
Marsman *et al*., 2004	LM plus DIA	49	Donor liver biopsy, FS	DIA software assessment	Pathologist with glass slide	Percentage of total fat, microvesicular and macrovesicular steatosis; correlation with liver function indices, graft and patient survival	Significant correlation between pathologist and software for macrovesicular steatosis and total fat; significant association of macrovesicular steatosis and graft survival both when assessed by pathologist or software
Niclauss *et al*., 2008	Static, stereo-microscope plus DIA	12	Pancreatic islets preparations	Computerized by 2 software and manual counting on digital images	Manual counting at microscope	Number, islet equivalents and purity of islet preparation	Total islet number, equivalents number, and purity were much better correlated between digital manual and computerized analyses than between standard manual and computerized analyses
Kissler *et al*., 2009	LM plus DIA	12	Pancreatic islets preparations	Computerized by software on digital image	Manual counting on digital image	Accuracy, intra- and inter-observer reproducibility for both modalities by means of CV	Digital image analysis is reliable for islet counting, with the advantage of permanent records and quality assurance
Biesterfield *et al*., 2012	Static LM, point grid counting	120	Donor liver biopsy, cut in half for FS and FFPE	Point grid counting	Conventional LM	Interobserver agreement for FS and FFPE, correlation between macro- and micro-vesicular steatosis	Substantial agreement (κ>0.60) and high correlation (r>0.80) between observers and types of steatosis; no advantage for point grid analysis

Contd...

Table 1: Contd...

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of the study	Results
Native *et al*., 2013	LM plus DIA	9 patients, 54 images	Donor liver biopsy	Model-based segmentation method algorithm	Expert pathologists with LM	Correlation between pathologists' assessments and automated image analysis-based evaluations of ld-MaS percentages	New algorithm proposed significantly improves separation between large and small macrovesicular lipid droplets (specificity 93.7%, sensibility 99.3%) and correlation with pathologists' ld-MaS percentage assessments (r=0.97)
Gymr *et al*., 2015	LM plus DIA	42	Pancreatic islets preparations	Automated by software on digital image	Manual counting at LM	Correlation of modalities for total islet number, equivalent number, and purity; intraobserver variability	High correlation between modalities for total islet and equivalent number; high intraobserver reproducibility for the use of software
Wang *et al*., 2015	LM plus DIA	25 patients, 84 samples	Pancreatic islets preparations	Computerized by software on digital image	Manual counting on digital image	Correlation of modalities for total islet number, equivalent number, and purity	Significantly high correlation between modalities; not significant difference for total counts
Mammas *et al*., 2015	Not clearly defined	518 images	Donor kidney, liver and pancreas	Diagnosis on digital image on 4 different viewing devices	Diagnosis of reference pathologist, not stated if with LM or digital	Accuracy of diagnosis with different viewing devices	The desktop and the experimental telemedicine platform are more reliable than tablet and mobile phone devices
Buchwald *et al*., 2016	LM plus DIA	3 patients, 14 samples	Pancreatic islets preparations	Computerized by software on digital image	Manual counting at LM	Correlation of modalities for total islet number, equivalent number, and purity; intraobserver variability	Very good overall correlation between modalities; lower intraobserver variability for DIA
Eccher *et al*., 2016	WSI	62 patients, 124 biopsies	Donor kidney wedge biopsy	Pathologist with WSI	Pathologist with glass slide	Intra- and inter-observer reproducibility with weighted Cohen k index	Very high intraobserver agreement (κ=0.961) for WSI and glass slide; slightly lower (κ=0.863) interobserver agreement for WSI than glass slide (κ=0.903)
Osband *et al*., 2016	Virtual microscope, not otherwise specified	23 kidneys	Donor kidney wedge biopsy, FS	Experienced pathologist with virtual microscope	On-site pathologist	Time to biopsy read	Shorter time to biopsy read with virtual microscope; improved time to local acceptance but not cold ischemia time or DGF rate
Liapis *et al*., 2017	WSI	40	Donor kidney biopsy	Experienced pathologist with WSI	None	Intraclass correlation coefficient for various parameters of score	Modest agreement among pathologist, only number of glomeruli, sclerosed glomeruli and interstitial fibrosis with ICC >0.5

Contd...

Table 1: Contd...

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of the study	Results
Cima *et al*., 2018	WSI	28	16 donor kidney wedge biopsy, FS 12 donor liver biopsy, FS	Scoring with WSI	Scoring with glass slide	Accuracy rate; intraobserver concordance with weighted Cohen k index; sensibility, specificity, PPV, NPV	86% accuracy rate, high intraobserver concordance (κ=0.91); 96%, 75%, 96%, 75% sensibility, specificity, PPV, NPV, respectively
Marsh *et al*., 2018	WSI	17 patients, 48 biopsy images	Donor kidney biopsy, FS	Patch-based model and fully convolutional model on WSI	Expert pathologist scoring with WSI	Comparison between the two models and with pathologist's assessment on WSI in counting total glomeruli and sclerosed glomeruli	Fully convolutional model substantially outperforming the model trained on image patches of isolated glomeruli, in terms of both accuracy and speed

CV: Coefficient of variation, DIA: Digital image analysis, FFPE: Formalin-fixed, paraffin-embedded, FS: Frozen section, LM: Light microscopy, ld-MaS: Large droplet Macrovesicular steatosis, NPV: Negative predictive value, PPV: Positive predictive value, WSI: Whole slide imaging, ICAM-1: Intercellular adhesion molecule-1, DGF: Delayed graft function, IHC: Immunohistochemistry, ICC: Islet cell counter

and adding value to the time-sensitive demand of donor biopsy evaluation. Their study is the first to specifically address glomerular recognition and classification in the FS preimplantation biopsy.[16]

The Banff group analyzed reproducibility among pathologists using WSI slides in a population of 40 donor kidney biopsies, with a different proportion of core versus wedge biopsies and FS versus paraffin technique. They reported overall good-to-excellent reproducibility for counting the total number of glomeruli, for assessing the percentage of sclerosed glomeruli and number of sclerosed glomeruli and interstitial fibrosis; however, the interobserver concordance was fair to poor in the assessment of other parameters.[25]

Osband *et al*. compared the time-to-donor kidney biopsy result between virtual microscopy and standard LM in practice and demonstrated a significant reduction in time-to-biopsy result using digital microscopy.[24] Mammas *et al*. compared the accuracy rate for the diagnosis of kidney, liver, and pancreas biopsies with a pathologist reading a digital slide on different devices, and they demonstrated that mobile phones and tablets to be less reliable than desktop viewing.[23] Finally, Benkoël *et al*. examined the expression of different IHC markers in a subset of paired preimplantation and postreperfusion liver biopsies, using DIA of confocal laser scanning microscope images, without comparison to conventional LM IHC.[14,19,20]

Digital pathology in post-transplantation

Among the 75 retrieved studies on posttransplant biopsies, 10 (13.5%) were concerned with liver biopsy, 16 (21.6%) with the heart and lung, and 47 (63.5%) kidney.

Liver graft biopsy

The studies concerning posttransplant liver graft biopsies are summarized in Table 2. Two studies[4,32] described the agreement with digital static pathology diagnosis and reported high concordance rates. Two more recent studies explored the reliability of WSI slides when compared to LM or reference diagnosis.[33,34] In the study by Neil *et al*., pathologists at several centers scored C4d antibody expression in liver biopsy tissue microarrays using WSI and LM. Interobserver agreement was variable with WSI when considering the different compartments of staining in a liver biopsy; in particular, concordance was good for the assessment of portal vein, central vein, and portal capillary compartments (κ = 0.60–0.80) and fair in the evaluation of sinusoidal and hepatic artery endothelium compartments (κ = 0.30–0.40). There was substantial agreement between pathologists with WSI and glass slides although κ indexes were not reported.[33] In the study of Saco *et al*., where WSI and LM were compared, the authors reported excellent intra- and inter-observer agreement (κ = 0.80–0.90) between modalities. Moreover, the authors highlighted the advantage of using WSI for viewing multiple slides, which is important because, in liver graft pathology, several stains are often used.[34]

Other studies regarding liver biopsy focused on the correlation with clinical parameters and predictive value on organ outcome for several features assessed by DIA software, such as fibrosis determined as collagen proportionate area (CPA) with Sirius red stain,[35-38] ductular reaction assessed with CK7 staining,[39] nuclear size, and IHC markers of oxidative damage.[40] In particular, CPA assessed as a continuous measure with DIA is reported to be a better predictor of graft outcome than Ishak stage assessed on conventional LM.[35-38] Ductular reaction area assessed with DIA software is reported to correlate with hepatic progenitor cell number assessed by manual counting and to be associated with hepatitis C virus (HCV) recurrence.[39] Nuclear size and anisonucleosis quantified with DIA software were not associated with any clinical parameters, except diabetes and the presence of a marker of oxidative damage.[40] Two older studies investigated the presence and role of overall inflammatory cells[41] and mast cells[42] for acute and chronic

Table 2: Summary of papers dealing with posttransplantation liver graft biopsy

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of study	Results
Ito *et al*., 1994	Static	22	Graft liver and kidney biopsy	Telepathology diagnosis	Direct LM diagnosis	Descriptive results	Agreement in 10/12 kidney biopsies and in 9/10 liver biopsies
Ben-Hari *et al*., 1995	LM plus DIA	55 (92 biopsies)	Graft liver biopsy	DIA assessment of eosinophil count, cell density and cross-sectional area in portal tract	None	Descriptive correlation of parameters with different degrees of rejection	Positive correlation of all parameters with severity of rejection
Minervini *et al*., 2001	Static	102, among which 9 liver graft and 9 kidney graft biopsies	Various case types: Second opinion consultation, transplantation pathology, general surgical pathology	Consultant telepathology review	Referring pathologist original diagnosis	Agreement rates, descriptive	86% agreement and 14% (only 3% major) disagreement between referring and consultant pathologist
El-Refaie *et al*., 2005	LM plus DIA	267 (343 biopsies)	Graft liver biopsy	DIA software quantification of mast cells and IHC staining	None	Correlation of mast cell count and IHC staining with different degrees of rejection	Strong correlation of mast cells with acute rejection and of IHC staining for c-Kit with severity of rejection
Calvaruso *et al*., 2008	LM plus DIA	115 (225 biopsies)	Graft liver biopsy	DIA software quantification of collagen proportionate area	None	Descriptive correlation between DIA measurements, Ishak score, and portal hypertension	Collagen proportionate area assessed by DIA correlated with Ishak stage scores and portal hypertension
Guzman *et al*., 2010	LM plus DIA	19 (33 biopsies)	Graft liver biopsy	Anisonucleosis and oxidative damage scored by DIA	None	Descriptive correlation of anisonucleosis with different clinical parameters	Higher anisonucleosis in individuals with diabetes and with high expression of oxidative damage marker
Manousou *et al*., 2011	LM plus DIA	135	Graft liver biopsy	Computer-assisted DIA quantification of collagen proportionate area	None	Descriptive correlation between DIA measurements, Ishak score, and decompensation	Collagen proportionate area assessed by DIA correlated with Ishak stage scores and decompensation
Calvaruso *et al*., 2012	LM plus DIA	65	Graft liver biopsy	Computer-assisted DIA quantification of collagen proportionate area	None	Descriptive correlation between DIA measurements, portal hypertension and graft outcome	Collagen proportionate area assessed by DIA correlated with portal hypertension and decompensation
Manousou *et al*., 2013	LM plus DIA	155 (587 biopsies)	Graft liver biopsy	Computer-assisted DIA quantification of collagen proportionate area and rate of increase	None	Descriptive correlation of DIA measurements and Ishak score with portal hypertension and graft outcome	Progression rate of fibrosis is a better predictor of clinical outcome than progression by Ishak stage
Sclair *et al*., 2016	LM plus DIA	60	Graft liver biopsy	DIA software assessment of ductular reaction in HCV recurrent recipients with cirrhosis	DIA software assessment of ductular reaction in stable recurrent HCV recipients with no cirrhosis or fibrosing hepatitis	Descriptive difference among the groups	Significantly higher ductular reaction in recipients with cirrhosis
Neil *et al*., 2017	WSI	40	TMAs of graft and native liver, kidney, heart	Pathologists scoring C4d with WSI	Pathologists scoring C4d with LM	Descriptive surveys of pathologists and comparison of staining methods	Strong and diffuse portal vein and capillary C4d staining, determined by both local and central pathologists, distinguished acute antibody-mediated rejection from native livers

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Table 2: Contd...

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of study	Results
Saco *et al*., 2017	WSI	64	Graft liver biopsy	Pathologist with WSI	Pathologist with LM	Intra- and inter-observer agreement	Almost perfect intraobserver concordance between modalities; high interobserver concordance for WSI (κ=0.80)

DIA: Digital image analysis, HCV: Hepatitis C virus, IHC: Immunohistochemistry, LM: Light microscopy, TMAs: Tissue microarrays, WSI: Whole-slide imaging

rejection, with quantification of cellular infiltrates or specific subtypes of mast cells with DIA software in digital images; they showed that the number of inflammatory cells assessed by DIA was able to separate mild from severe rejection[41] and that mast cell density both with tryptase and c-Kit staining correlated with the severity of acute and chronic rejection.[42]

Heart and lung graft biopsy

The studies concerning posttransplant heart and lung graft biopsies are summarized in Table 3. Of papers concerning heart and lung graft biopsy, 2/16 (12.5%) dealt with agreement and reproducibility between digital slides and LM. The oldest study by Marchevsky *et al*. reported concordance rates of 96% and 82.8% with Cohen's κ coefficients of 0.92 and 0.692 for lung and heart biopsy, respectively. Using static digital pathology, images were acquired with a camera attached to a microscope, remotely diagnosed by a pathologist, and then compared to a reference diagnosis.[43] A more recent study by Angelini *et al*. reported fair interobserver concordance among pathologists (κ = 0.20–0.40) when assessing a set of 20 endomyocardial biopsies (EMBs). The interobserver agreement increased when pathologists were stratified according to their expertise in heart transplant pathology.[44]

Most of the studies (9/16, 56.3%) dealt with graft rejection and quantification of parameters that aid in grading the severity of rejection or help elucidate potential pathogenetic mechanisms. Features quantified with DIA software included myocyte diameter,[45] fibrosis with Masson's trichrome stain,[45,46] microvasculature density with CD31[46] or CD34,[47] patterns of inflammatory and immunological cells,[48] monocytes and macrophage profiles,[49] expression of Sirt1, CD8, and FoxP3 on lymphocytes in rejection specimens,[50] and chromatin remodeling expressed as mean gray level.[51] In some publications, digital images were converted in formats adequate for fractal analysis to quantify the inflammatory infiltrate and signs of myocyte damage; it was shown that this kind of DIA can discriminate among different grades of rejection.[52,53] Other parameters assessed on graft biopsy with DIA software on LM images (nuclear parameters of cardiomyocytes[54] or fibrosis with Azan-Mallory stain and microvascular remodeling with IHC staining[55]) were relevant for recipient outcome of different immunosuppressive treatments. Overall, the quantitative assessment of EMBs by means of DIA provided more information than routine, semi-quantitative investigation, even if the application of DIA software required a more reproducible staining quality among slides and a better than routine quality of histological slides.[54] Image analysis was also used to quantify macrophages and T-lymphocytes in autopsy specimens of coronary vessels of transplanted heart recipients to compare several vascular remodeling features.[56] Finally, only two studies concerned lung biopsies and both explored the correlation of basement membrane thickness measured with DIA software with the development of bronchiolitis obliterans in recipients. They found that increased thickness of the basement membrane can be transient and not correlated to respiratory function decline.[57,58] For the majority of the aforementioned studies, DIA was carried out on static digital images acquired with an LM. Only three out of 14 studies where DIA was employed used WSI technology. This is not surprising given that WSI adoption was only adopted more recently.

Kidney graft biopsy

The studies concerning posttransplant kidney graft biopsies are summarized in Table 4. Articles concerning the posttransplantation kidney biopsy were the most numerous (47/75, 62.7%) and dealt with various topics. Apart from the studies by Minervini *et al*.[4] and Ito *et al*.[32] that also included kidney biopsies, nine out of 47 studies (19.1%) addressed agreements between LM and digital slide assessment for several parameters.[59-67] Ito *et al*. used a static telepathology system and only evaluated the concordance rate,[59] while more recent studies used WSI and achieved good or substantial (κ > 0.40 and κ > 0.60) intra- and inter-observer agreements, concluding that WSI is as reliable as LM for graft biopsy evaluation.[64,65] Older studies used LM plus DIA software for the quantification of fibrosis, inflammation, and glomerular sclerosis, reporting that DIA assessment had good correlation with manual evaluation, but that it had higher correlation with graft outcome.[60-62] More recent studies combining WSI with DIA for the quantification of C4d IHC,[63] fibrosis with PAS staining and collagen IHC,[66] and CD3 for acute rejection[67] showed that digital evaluation had better correlation with organ function and higher reproducibility than LM assessment.[63,66,67]

Most of the studies on graft kidney biopsy use DIA techniques to explore the role of several biopsy features ranging from fibrosis evaluated with special stains to the expression and quantification of specific IHC markers in determining organ outcome,[68-83] as well as signs of acute rejection.[84-93] In all of

Table 3: Summary of papers dealing with posttransplantation heart and lung graft biopsy

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of the study	Results
Armstrong *et al*., 1998	LM plus DIA	101	EMBs	DIA software assessment of fibrosis and myocyte diameter in recipients	DIA software assessment of fibrosis and myocyte diameter in controls	Descriptive differences between the groups	Larger myocyte diameter in transplanted hearts; fibrosis higher in the first posttransplant EMBs
Marchevsky *et al*., 2002	Static LM	108	Graft lung and heart biopsy	Telepathology diagnosis	Previous LM diagnoses	Agreement rates, descriptive	96% agreement, κ=0.92, for lung biopsies, 82.8% agreement, κ=0.692, for EMBs
Law *et al*., 2005	LM plus DIA	25	Graft lung biopsy	DIA software quantification of basement membrane thickness	None	Correlation of basement membrane thickness with the development of bronchiolitis obliterans syndrome	Strong negative correlation of basement membrane thickness versus time
Ward *et al*., 2005	LM plus DIA	30 (21 biopsies)	Graft lung biopsy	DIA software assessment of basement membrane thickening	Published data on basement membrane thickening in other lung diseases	Descriptive results in lung recipients and correlation with respiratory function parameters	Higher basement membrane thickening compared to published data in other lung diseases; no correlation with lung function
Sorrentino *et al*., 2006	LM plus DIA	21 (361 biopsies)	EMBs	DIA of IHC staining	None	Descriptive	Role of IHC assessment in grading rejection
Zakliczynski *et al*., 2006	LM plus DIA	43 (129 biopsies)	EMBs	Automated software quantification of nuclei	None	Descriptive	Role of chromatin distribution in nuclei to assess severity of rejection
Nozynski *et al*., 2007	LM plus DIA	31	EMBs	Use of ATG	Standard treatment	Descriptive differences in quantitative assessment of nuclear parameters with automated software in the groups	Nuclear parameters of rejection lower in the ATG group
Angelini *et al*., 2011	WSI	20	EMB	18 pathologists reading WSI slides	Index diagnosis of referent pathologist	Interobserver reproducibility and agreement with reference	Fair-to-moderate reproducibility (κ=0.39, α=0.55); role of expertise for agreement with reference diagnosis
Moreira *et al*., 2011	LM plus DIA	Not stated, 658 images	EMBs	Fractal dimension by DIA software	None	Descriptive relation between fractal dimension and degrees of rejection	Fractal dimension can discriminate between degrees of rejection
Revelo *et al*., 2012	WSI plus DIA	22	EMBs	Microvessel density in recipients with AMR	Microvessel density in recipients without AMR	Descriptive	Significantly reduced microvessel density in a subset of patients with pathologic AMR with worse outcome
Devitt *et al*., 2013	LM plus DIA	34	Transplanted hearts in deceased recipients	Measurement on acquired images	None	Descriptive	Consideration of donor-derived accelerated atherosclerosis in heart recipients
Pijet *et al*., 2014	LM plus DIA	40	EMBs	Fractal parameters assessment with DIA software	None	Descriptive differences between grades of rejection	Some digital parameters can aid grading of rejection

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Table 3: Contd...

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of the study	Results
Tona *et al*., 2014	LM plus DIA	28	EMBs	Everolimus	Mycophenolate mofetil	Difference in fibrosis, microvascular remodeling, and arteriolar thickening	Capillary density and fibrosis comparable between groups, arteriolar thickening lower in the everolimus group
Welsh *et al*., 2016	LM plus DIA	13	EMBs	DIA software assessment of IHC staining	None	Evaluation of Sirt-1 expression in acute cellular rejection	Increased expression of Sirt-1 in lymphocytes in acute cellular rejection
Feingold *et al*., 2017	WSI plus DIA	9	EMB with LGD	EMBs with WSI	9 matched control EMBs with WSI	Automated quantification of fibrosis and microvascular changes	Greater fibrosis and microvascular changes in LGD cases
Van den Bosch *et al*., 2017	WSI plus DIA plus confocal microscopy	25 (50 EMBs)	EMBs	EMBs at time of rejection	EMBs at no rejection time	Difference in monocyte and macrophage infiltration and degree of fibrosis	CD16+monocyte, M2 macrophage infiltration, and higher fibrosis are associated with rejection

ATG: Anti-thymocyte globulin, DIA: Digital image analysis, EMBs: Endomyocardial biopsies, IHC: Immunohistochemistry, LGD: Late graft dysfunction, LM: Light microscopy, WSI: Whole-slide imaging, AMR: Antibody-mediated rejection

these studies, there is no direct comparison of DIA evaluation with manual pathologist results. Moreover, most of these are retrospective or case–control observational studies. The most studied parameter was interstitial fibrosis, with the correlation of DIA quantitative assessment to organ outcome being the main focus of these studies. Interstitial fibrosis was highlighted with special stains or with IHC, and some studies included comparison with other techniques such as spectroscopy[74] or Doppler ultrasound for renal resistance index.[81] Even though organ outcome was assessed slightly differently, most of these studies reinforced the idea that precise and automated quantification of this parameter by DIA technique can add value to biopsy evaluation, providing more reproducible results and permitting comparisons to be made with findings from other researchers. Similarly, studies about rejection mostly compared the IHC expression of several inflammatory markers and immune system cellular infiltration evaluated with DIA software in rejection biopsies and normal control biopsies. The remaining studies on posttransplantation kidney biopsy explored other features that correlated with ischemic injury,[94] levels of glomerular sclerosis,[95] fibrosis in grafts from after-brain-death donor or cardiac-death donor,[96] IHC markers to quantify interstitial fibrosis,[97-99] correlation with Banff score parameters[100] and more subtle features such as swollen glomerular epithelial cells.[101] Finally, three studies from the same research group compared fibrosis, assessed with special stains or IHC, and quantified by DIA software, in patients receiving cyclosporine or tacrolimus.[102-104]

Two main research themes: concordance and correlation to outcome

As already mentioned, the main issues addressed overall were the concordance between standard LM or manual assessment and WSI or DIA instruments and the correlation of histological features assessed by DIA methods with the outcome. The first topic was the most frequent in pretransplant papers. Intra- and inter-observer concordance with κ index was high when comparing WSI with LM,[15,26] thus reinforcing the point that digital diagnosis could replace conventional glass-slide diagnosis. The group of studies concerning pancreatic islet counting,[27-31] even with slightly different statistical measures, however, pointed toward the same direction, stating that DIA assessment is highly correlated to manual standard assessment and had the advantage of lesser interoperator variability. This remained true also in posttransplant papers addressing the same topic, even if less numerous.[33,34,44,63-67] In particular, more recent studies combining DIA with WSI concluded that DIA assessment of features has not only higher reproducibility than LM but also a better correlation to graft outcome, thus embracing with the second more frequent topic encountered through papers. This applies particularly to liver and kidney graft pathology, where a quota of papers compared DIA to manual assessment of features on LM-digitized images and correlated to outcome. With different grade of strength, they all suggested a better correlation to outcome and the advantage of a higher reproducibility. However, the vast majority of these studies were retrospective, both in the case of only concordance/reproducibility studies and of correlation-to-outcome studies, with the use of archival cases where the reference diagnosis was made previously with LM and sometimes with partly overlapping case populations.[35-38] Even if a quality assessment of studies was beyond the aims of this work, it is noticeable that only few studies were multicentric with the involvement of pathologists not working together, thus minimizing possible bias.[33,44,66] Moreover,

Table 4: Summary of papers dealing with posttransplantation kidney graft biopsy

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of the study	Results
Ito *et al*., 1994	Static LM	22	Graft liver and kidney biopsy	Telepathology diagnosis	Direct LM diagnosis	Descriptive results	Agreement in 10/12 kidney biopsies and in 9/10 liver biopsies
Gandaliano *et al*., 1997	LM plus DIA	20	Graft kidney biopsy	DIA assessment of IHC staining for CD68 and MCP-1 in acute rejection biopsies	DIA assessment of IHC staining for CD68 and MCP-1 in tubular damage and control biopsies	Descriptive differences in expression between groups and correlation with graft outcome	MCP-1 expression significantly higher in acute rejection biopsies
Grimm *et al*., 1999	LM plus DIA	32	Graft kidney biopsy	DIA assessment of IHC staining of cellular infiltrate in clinical and subclinical rejection biopsies	DIA assessment of IHC staining of cellular infiltrate in normal controls	Descriptive differences in IHC staining among the groups	Significantly higher infiltration of CD8 and CD68 positive cells in clinical rejection
Nicholson *et al*., 1999	LM plus DIA	52	Graft kidney biopsy	Semiautomatic DIA assessment of interstitial fibrosis with IHC	None	Descriptive correlation of interstitial fibrosis with graft outcome	Positive correlation of interstitial fibrosis as stained area with eGFR
Bonsib *et al*., 2000	LM plus DIA	14 (42 biopsies)	Graft kidney biopsy	Tubular membrane breaks with methenamine silver assessed on digital images	None	Descriptive correlation with clinical parameters	Correlation of tubular membrane breaks with creatinine level
Furukuwa *et al*., 2001	LM plus DIA	21	Graft kidney biopsy	DIA software assessment of interstitial fibrosis	None	Descriptive correlation of degree of interstitial fibrosis with graft outcome	Usefulness of the computerized imaging diagnosis for quantitative evaluation of interstitial fibrosis in predicting graft failure
Ishimura *et al*., 2001	LM plus DIA	21	Graft kidney biopsy	DIA software assessment of interstitial fibrosis	None	Descriptive correlation between interstitial fibrosis and TGF=beta IHC staining	Strong association between extracellular TGF beta expression and long-term decline in graft function and increased interstitial fibrosis
Ito *et al*., 2001	Static LM	31 (37 biopsies)	Graft kidney biopsy	Telepathology diagnosis	Direct LM diagnosis	Descriptive results	Agreement on diagnosis in 30/37 cases
Minervini *et al*., 2001	Static LM	102	Various case types, among which 9 kidney graft biopsies	Consultant telepathology review	Referring pathologist original diagnosis	Agreement rates, descriptive	86% agreement and 14% (only 3% major) disagreement between referring and consultant pathologist
Danilewicz *et al*., 2003	LM plus DIA	34	Graft kidney biopsy	DIA assessment of IHC staining and glomerular area in biopsies with acute rejection	DIA assessment of IHC staining and glomerular area in normal controls	Descriptive differences in IHC staining between the two groups	Significantly higher cellular infiltrate, glomerular area and interstitial area in acute rejection biopsies
Encarnacion *et al*., 2003	LM plus DIA	49	Graft kidney biopsy	Different computerized strategies of DIA	Expert pathologist with LM	Correlation of tubulointerstitial fibrosis with graft function	Different degree of correlation with graft function of tubulointerstitial fibrosis scored with different strategies
Grimm *et al*., 2003	LM plus DIA	NA	Graft kidney biopsy	Automated DIA software assessment of interstitial fibrosis	None	Correlation of interstitial fibrosis with graft outcome	Cortical fractional interstitial fibrosis volume can be a surrogate for time to graft failure
Mui *et al*., 2003	LM plus DIA	30	Graft kidney biopsy	DIA assessment of IHC staining in ischemic injury	DIA assessment of IHC staining in normal controls	Descriptive	Different pattern of expression of markers in ischemic injury biopsies

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Table 4: Contd...

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of the study	Results
Pape *et al*., 2003	LM plus DIA	56	Graft kidney biopsy	DIA assessment of interstitial fibrosis	None	Correlation of interstitial fibrosis with graft outcome	Quantitative measurement of fibrosis by picrosirius red staining is a prognostic indicator for estimating long-term graft function
Sugiyama *et al*., 2003	LM plus DIA	25	Graft kidney biopsy	DIA assessment of mean glomerular area and interstitial area	None	Descriptive differences in recipients with or without focal segmental glomerulosclerosis	No significant difference in mean glomerular area nor interstitial area between the two groups
Bains *et al*., 2004	LM plus DIA	112	Graft kidney biopsy	DIA software assessment of fibrosis in DCD and DBD graft biopsies	None	Difference of fibrosis in the two groups	No significant differences in level of fibrosis
Danilewicz *et al*., 2004	LM plus DIA	35	Graft kidney biopsy	DIA quantification of mast cells and leukocytes with IHC staining in acute rejection biopsies	DIA quantification of mast cells and leukocytes with IHC staining in normal controls	Descriptive differences between the groups	Significantly higher number of mast cells and leukocytes in acute rejection; positive correlation between inflammatory infiltrate and interstitial area
Pape *et al*., 2004	LM plus DIA	56	Graft kidney biopsy	Renal resistance index with Doppler	Interstitial fibrosis assessment with DIA	Correlation between the two measurements and with graft outcome	Positive correlation between the two measures and of the combination of the two with graft outcome
Sarioglu *et al*., 2004	LM plus DIA	15	Graft kidney biopsy	Automated quantification of stained area	None	Descriptive	Strong correlation between stained area and serum creatinine (r=0.64)
Sund *et al*., 2004	LM plus DIA	33	Graft kidney biopsy	DIA automated quantification	Pathologist with LM	Descriptive	Significant correlation between the two modalities and with graft outcome
Nishi *et al*., 2005	LM plus DIA	14	Graft kidney biopsy	DIA software assessment of the peritubular capillary network in recipients with rejection	DIA software assessment of the peritubular capillary network in recipients without rejection	Descriptive	Significant differences in surface areas of tubulin and glomerular diameter between the groups
Sis *et al*., 2005	LM plus DIA	57 (75 biopsies)	Graft kidney biopsy	DIA software assessment of stained area	None	Descriptive correlation among stained areas for fibrosis, Banff scores and rejection	No significant association between serum creatinine at time of biopsy and percentage of stained areas for fibrosis; no predictive value for rejection
Danilewicz *et al*., 2006	LM plus DIA	33	Graft kidney biopsy	DIA of IHC staining in acute rejection recipients	DIA of IHC staining in recipients with no rejection	Differences in IHC staining in the two groups	Higher expression of TGF beta, CD3, CD8 in acute rejection
Hoffman *et al*., 2006	LM plus DIA	138	Graft kidney biopsy	DIA of IHC staining	None	Descriptive expression of CXCR3	Higher expression of CXCR3 in acute rejection
Lauronen *et al*., 2006	LM plus DIA	35	Graft kidney biopsy	DIA software scoring	Pathologist with LM	Descriptive	No significant difference in scoring between the modalities
Roos-van-Groningen *et al*., 2006	LM plus DIA	54 (108 biopsies)	Graft kidney biopsy	Cyclosporine	Tacrolimus	Fibrosis and IHC staining assessed by automated DIA software	No quantitative differences in fibrosis and IHC staining between cyclosporine and tacrolimus

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Table 4: Contd...

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of the study	Results
Rowshani *et al*., 2006	LM plus DIA	126	Graft kidney biopsy	Cyclosporine	Tacrolimus	Fibrosis with Sirius red assessed by automated DIA software	No difference in the degree of interstitial stained area between the two treatment groups
Sarioglu *et al*., 2006	LM plus DIA	37 (44 biopsies)	Graft kidney biopsy	DIA assessment of periodic acid methenamine silver staining	None	Descriptive relation of stained area to Banff scores and creatinine values	Strong association of stained area with increased interstitial fibrosis and tubular atrophy Banff scores
Scholten *et al*., 2006	LM plus DIA	126	Graft kidney biopsy	Cyclosporine	Tacrolimus	Subacute rejection assessed by pathologist and automated fibrosis quantification	No quantitative differences in fibrosis between cyclosporine and tacrolimus; higher prevalence of subacute rejection in the cyclosporine group but no difference in graft survival
Servais *et al*., 2007	LM plus DIA	26	Graft kidney biopsy	DIA automated quantification of interstitial fibrosis in recipients treated with cyclosporine	None	Descriptive correlation of interstitial fibrosis with graft outcome	Correlation of higher grade of automated interstitial fibrosis with a higher creatinine
Servais *et al*., 2007	LM plus DIA	26	Graft kidney biopsy	DIA automated quantification of interstitial fibrosis in recipients treated with cyclosporine	None	Descriptive correlation of interstitial fibrosis with graft outcome	Association between high grade of automated interstitial fibrosis and worsening of creatinine clearance
Birk *et al*., 2010	LM plus DIA	29 (105 biopsies)	Graft kidney biopsy	DIA software quantification of interstitial fibrosis	None	Descriptive correlation of interstitial fibrosis and graft outcome	Significant correlation of interstitial fibrosis assessed by DIA software with graft outcome
Yan *et al*., 2010	LM plus DIA	46	Graft kidney biopsy	DIA quantification of IHC staining	None	Correlation of IHC staining with Banff score for interstitial fibrosis and tubular atrophy	Higher IHC staining expression in higher Banff score classes for interstitial fibrosis and tubular atrophy
Brazdziute *et al*., 2011	WSI plus DIA	32 (34 biopsies)	Graft kidney biopsy	Automated software on WSI	Pathologist on LM	Correlation and interobserver variability in C4d scoring	Good-to-high correlation between pathologist and automated software; good manual-automated interobserver agreement
Meas-Yedid *et al*., 2011	WSI plus DIA	90 biopsies	Graft kidney biopsy	Automated software on WSI	Expert pathologist on LM	Correlation and interobserver variability in interstitial fibrosis scoring	Good agreement between the two methods (κ=0.75)
Miura *et al*., 2011	LM plus DIA	109	Graft kidney biopsy	DIA software assessment of interstitial fibrosis	None	Correlation of interstitial fibrosis different tacrolimus regimens and cytochrome polymorphism	Higher increase in interstitial fibrosis in absence of cytochrome polymorphism
Servais *et al*., 2011	LM plus DIA	140	Graft kidney biopsy	Automated DIA software assessment of interstitial fibrosis	None	Correlation of interstitial fibrosis with graft outcome	Correlation between interstitial fibrosis at different time points and eGFR

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Table 4: Contd...

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of the study	Results
Becker *et al*., 2012	LM plus DIA	40	Graft kidney biopsy	IHC staining in cellular infiltrate of clinical, operational tolerance recipients	IHC staining in cellular infiltrate of rejection recipients	Descriptive expression of IHC staining in inflammatory infiltrate	Different IHC staining in the two groups
Ozluk *et al*., 2012	WSI	40	Graft kidney biopsy	Pathologists with WSI	Pathologists with LM	Intra- and inter-observer reproducibility	Comparable intraobserver reproducibility for both modalities; higher interobserver reproducibility with WSI
Yan *et al*., 2012	LM plus DIA	28	Graft kidney biopsy	DIA software quantification of IHC staining of GSK3 beta at different levels of inflammation	None	Descriptive correlation between GSK3 beta staining and inflammation	Stronger GSK3 beta expression with increasing grade of inflammation or interstitial fibrosis/tubular atrophy
Yan *et al*., 2012	LM plus DIA	61	Graft kidney biopsy	DIA software quantification of IHC staining in recipients with AMR	DIA software quantification of IHC staining in recipients without AMR	Descriptive relationship of IHC staining of extracellular matrix cytokines with interstitial fibrosis and creatinine	Higher expression in grafts with AMR; increasing expression with higher Banff scores of interstitial fibrosis and positive correlation with creatinine
Caplin *et al*., 2013	LM plus DIA	246	Graft kidney biopsy	Serial posttransplant biopsies	No serial biopsies	Descriptive correlation of index of chronic damage with graft function	No significant differences between the two groups; index of chronic damage not predictive of graft function
Jen *et al*., 2013	WSI	25	Graft kidney biopsy	Expert pathologists with WSI	Expert pathologist with LM	Intra- and inter-observer concordance	Substantial intraobserver concordance between modalities (κ=0.60), moderate interobserver concordance (κ=0.41-0.45)
Farris *et al*., 2014	WSI plus DIA	30	Graft kidney biopsies	Pathologists scoring interstitial fibrosis on WSI slides with different stains	Computerized DIA of collagen IHC staining	Interobserver reproducibility and correlation of visual assessment on WSI with DIA assessment and with graft outcome	Poor reproducibility between pathologists; moderate correlation of visual assessment with DIA assessment of collagen-IHC; moderate correlation with graft outcome with no significant differences between the modalities
Vuiblet *et al*., 2015	LM plus DIA plus spectroscopy (FTIR)	106 (166 biopsies)	Graft kidney biopsy	Spectroscopy	Pathologist with LM and DIA	Quantification of interstitial fibrosis and inflammation	Poor agreement between scoring LM versus DIA and LM versus FTIR, good agreement in percentages between DIA and FTIR; good correlation between fibrosis with FTIR and graft function
Hara *et al*., 2016	LM plus DIA	934	Graft and native kidney biopsy	426 graft biopsy	508 native kidney biopsy	Quantification of GSECs	Prevalence of GSECs slightly increased with posttransplant duration but not statistically significant
Yan *et al*., 2016	LM plus DIA	50	Graft kidney biopsy	DIA software assessment of IHC staining in graft with chronic dysfunction	DIA software assessment of IHC staining in graft with no dysfunction	Difference in markers expression and correlation with Banff scores for interstitial fibrosis/tubular atrophy	Higher expression in grafts with dysfunction; positive correlation between marker expression and Banff scores

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Table 4: Contd...

Author, year	Type of digital pathology	Number of patients/ biopsies	Type of biopsy	Intervention	Controls or comparisons	Outcomes/Aim of the study	Results
Bräsens *et al*., 2017	WSI plus DIA	67	Graft kidney biopsy	Automated software on WSI	None	Correlation of different cellular types digitally quantified with graft function	Predictive value of digitally quantified CD68 cell density for graft function
Moon *et al*., 2017	WSI plus DIA	45	Graft kidney biopsy	DIA automated software assessment of interstitial inflammation with different algorithms	Visual assessment of interstitial inflammation	Descriptive correlation among the modalities	Quantitation algorithms correlated between each other and also with visual assessment

AMR: Antibody-mediated rejection, DBD: Donor after brain death, DCD: Donor after cardiac death, DIA: Digital image analysis, eGFR: Estimated glomerular filtration rate, FTIR: Fourier-transformed infrared spectroscopy, GSECs: Granular swollen epithelial cells, IHC: Immunohistochemistry, LM: Light microscopy, WSI: Whole-slide imaging, MCP-1: Monocyte chemotactic peptide-1, TGF: Transforming growth factor

in the majority of studies, digital pathology pertained only to the research field, especially in case of assessment of histological features or particular IHC marker expression, but also for concordance studies, where the value of digital pathology is explored in view of a possible future clinical full implementation.

Conclusion and Future Directions

The aim of this review was to provide a broad overview of accrued international experience in the use of digital pathology in transplantation. Most retrieved studies involved the evaluation of the posttransplantation biopsy. The acquisition, manipulation, and eventual transmission of digital slides, before the advent of WSI, were too slow to be compatible with the time-sensitive needs encountered in the preimplantation setting. DIA was more adequate for outcome studies where time is not necessarily an issue.

It is not surprising that most of the studies using WSI, in particular, those in the pretransplant context, focused on the diagnostic agreement and concordance between LM and WSI. Indeed, it is likely that WSI may soon replace conventional LM diagnosis, especially as newer generation scanners acquire higher resolution images and digital platforms facilitate easier sharing of digital slides among pathologists. Some conventional barriers to implementation of WSI such as costs and storage issues could now be overcome in big centers and academic institutions. Some questions remain open, mainly concerning the regulatory constraints in different countries and economic issues on payer/reimbursement that apply particularly to the transplantation setting, for example, for second-opinion consultations and quality control programs, as transplantation activity is traditionally managed by public national health system.

The number of studies about WSI coupled with DIA is relatively small and restricted to the last 8 years. However, it is foreseeable that in the future, there will be a growing number of studies applying DIA and most likely deep learning algorithms and artificial intelligence to WSI, thereby augmenting the practice and field of transplantation.[8]

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Conflicts of interest

There are no conflicts of interest

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