Digital Slide and Simulation-Based Learning in Pre-Implantation Kidney Biopsies

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Abstract

Although controversial, procurement kidney biopsies and histology are commonly used in kidney allocation from deceased donors. The long series of models developed for this question, incorporating a variety of clinical and histologic variables, failed to properly predict the long-term graft survival. This failure could be explained by many factors, including the lack of expertise in terms of skilled available nephropathologists in the urgent setting of biopsies assessment. Simulation-based learning is a form of experiential learning that provides learners with a real-world-like opportunity to develop and practice their knowledge and skills but in a simulated environment. Digital pathology with whole-slide imaging is a powerful tool for knowledge delivering, as it offers the opportunity to facilitate meeting of general pathologists with experts, with availability of second opinion consultation and tailored training on specific cases. In the back of these considerations, we report on the content of the web-meeting "Digital slide and simulation-based learning in pre-implantation kidney" which was fully dedicated to the evaluation of pre-implantation kidney biopsy, with a very practical approach and a direct interaction between two expert renal transplant pathologists and the audience of general pathologists.

Keywords Pre-implantation kidney biopsies · Digital pathology · Simulation-based learning · Transplantation

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Introduction

For patients in end-stage renal disease, renal transplantation is the therapy of choice, as it improves patients' survival and quality of life, but demand for organs is still higher than the offer. Therefore, it is of paramount importance to avoid inappropriate organ discard and maximize retrieval also from extended criteria donors (ECD). Pre-implantation biopsy is usually undertaken to evaluate the suitability of the kidneys in ECDs, although there are still many challenges in the management and assessment. Apart from the modality of bioptic sampling (wedge versus needle core vs the recently introduced punch biopsy), there is conflicting evidence on which histological parameters best represent the possible outcome of the transplantation [1]. Kidney biopsy for organ suitability is usually assessed with Remuzzi/Karpinski scores, or slight modifications of them, and these comprise evaluation of glomerulosclerosis (GS), interstitial fibrosis (IF), tubular atrophy (TA), and arterial arteriosclerotic narrowing [2]. To complicate things, kidney biopsies for organ suitability are usually evaluated by on-call pathologists with no specific

expertise in renal pathology, and published evidence has highlighted that expertise and training have great influence on final score of biopsy and therefore potential discard or inappropriate allocation, with different correlation to final outcome [3, 4••]. Moreover, detection of subtle alterations in kidney such as thrombotic microangiopathy or tubular necrosis or grading diabetic damage which can have a great influence on graft function in the recipient can be very challenging for general pathologists with no specific expertise in this specific field.

Digital pathology with whole-slide imaging (WSI) has shown to be reliable for transplant biopsy evaluation and in general for transplant services [5–8]. Moreover, it is a powerful instrument for knowledge delivering, as it offers the opportunity to facilitate meeting of general pathologists with experts, with availability of second opinion consultation and tailored training on specific cases [9•].

In the back of these considerations, we report on the content of the web meeting "Digital slide and simulation-based learning in pre-implantation kidney," held on Zoom platform on January 20, 2023 (Fig. 1). The meeting was fully dedicated to the evaluation of pre-implantation kidney biopsy, with a very practical approach and a direct interaction between two expert renal transplant pathologists and the audience of general pathologists and nephrologists. Several pathognomonic and challenging cases of kidney biopsies from real-life practice were explained and discussed by the experts as the main goal of this meeting was to offer a permanent access to this piece of knowledge for all the reading pathologists involved in pre-implantation kidney biopsy evaluation. Before starting the discussion, the experts reported their experience on how to procedurally manage each single case, with periodic acid Schiff (PAS) and trichrome stains recommended for a correct and easier identification of structures and alterations in pre-transplant kidney biopsy.

Meeting Overview

A total of 53 slides referring to 22 different cases were scanned with 3DHisthec Pannoramic scanner to a maximum magnification of $40\times$ and uploaded on a dedicated platform, where they were available to all the attendees before and after the meeting. File size ranged from 108.83 MB to 1.6 GB, and for most of the cases, both a PAS and a trichrome stain were available, sometimes together with other additional special and immunohistochemical stains. The cases have been divided and discussed into specific sections starting from basics on how to evaluate adequacy to most challenging cases.
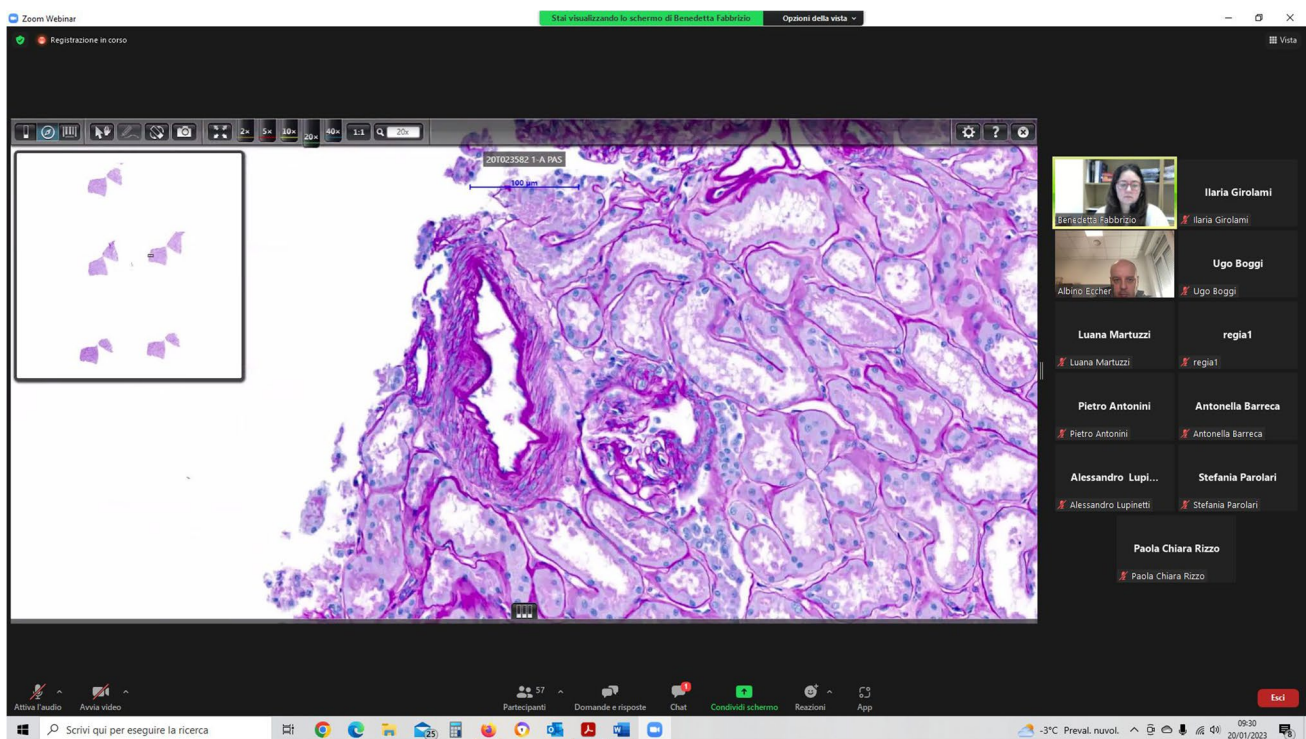

Fig. 1 Web meeting on "Digital slide and simulation-based learning in pre-implantation kidney," held on Zoom platform on January 20, 2023
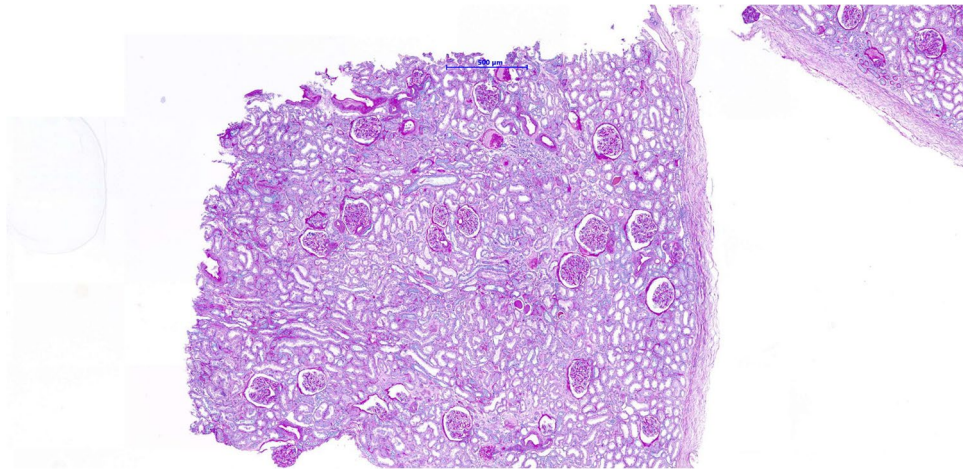
Back to Basics (Figs. 2, 3, 4, and 5)

Fig. 2 A low power overview of a punch biopsy of kidney with PAS staining. Adequacy of a biopsy is given when at least 25 glomeruli and 2 arteries are present and evaluable. Capsular surface is on the right here; on the left side, an arcuate artery at the border with medulla is seen, and the experts explained that all the sclerotic glomeruli and fibrotic areas found near the capsular surface should not be considered for the purpose of the Remuzzi/Karpinski score, because this area is affected by subtle chronic ischemic damage even in normal kidneys, and the risk is to overestimate the overall amount of damage. PAS, 3×magnification

Fig. 3 In this 20×magnification, we appreciate the basic structures of kidney parenchyma with mild alterations. The glomeruli are mostly healthy with empty capillary lumens and small arteriole at their pole (red stars), while in the middle, a completely sclerotic glomerulus is seen (black star). An artery is seen below a glomerulus, and on the left, a mild area of fibrosis and tubular atrophy is perceptible. PAS, 20×magnification
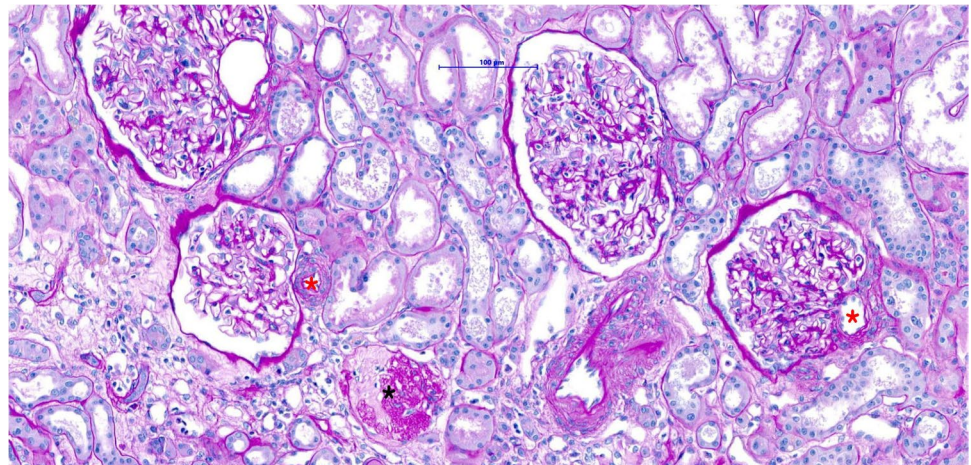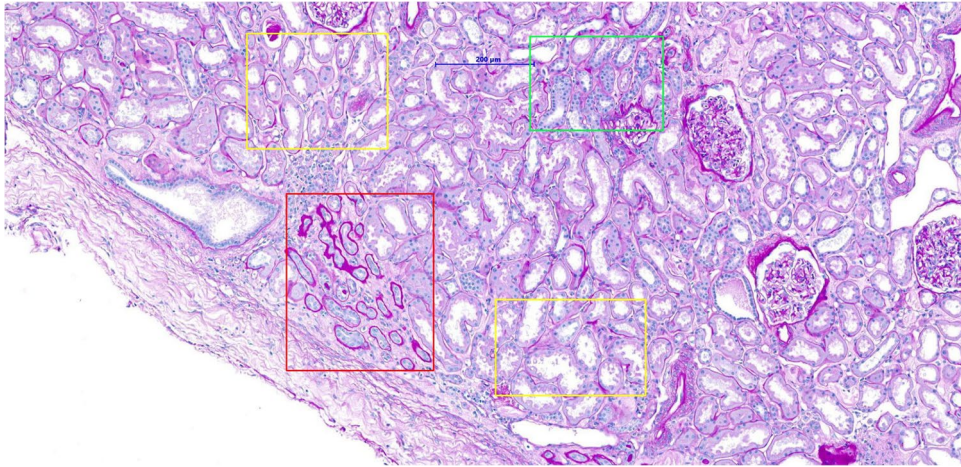
Fig. 4 In this 10× magnification, the difference between proximal and distal tubuli and between normal and atrophic tubuli is highlighted. Proximal tubuli have the brush border in the lumen and a slightly larger cytoplasm (yellow boxes), while distal tubuli have relatively more packed cells, resembling ductal biliary structures (green box). Atrophic tubuli have a thick PAS-stained basement membrane (red box) and are placed in an area of interstitial stromal expansion. The score for tubular atrophy and interstitial fibrosis is very often the same, and in Banff score for native kidney, they represent a unique compartment. PAS, 10× magnification

Fig. 5 In this 40× magnification, atrophic tubuli stands clearly apart from a majority of proximal tubuli with clearly discernible brush border and mild acute damage. PAS, 40× magnification
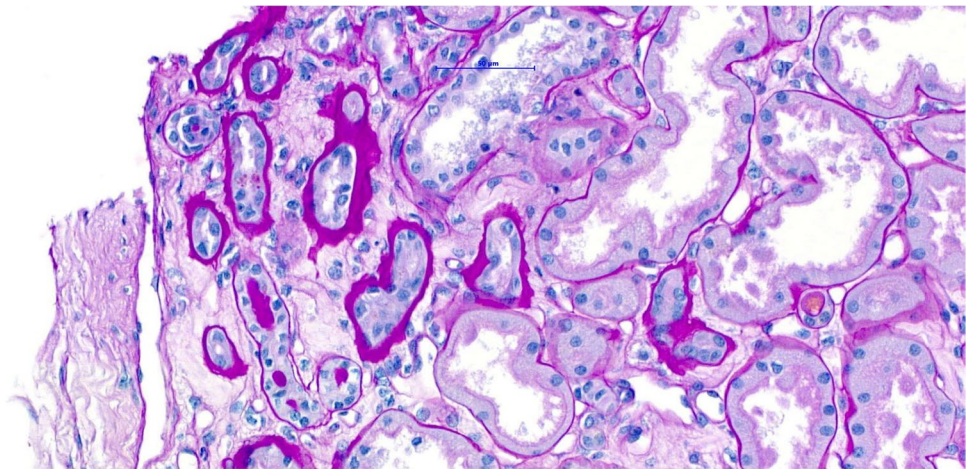
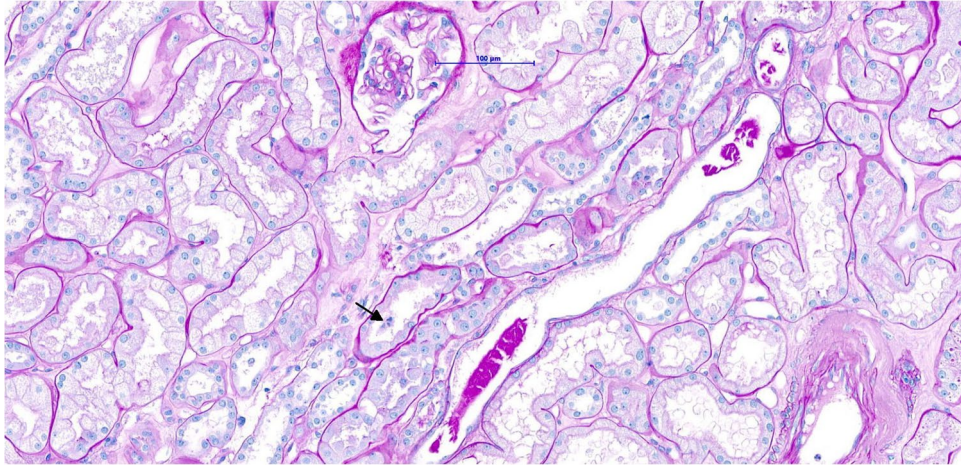# Signs of Acute Damage (Figs. 6, 7, and 8)

Fig. 6 In this 20×magnification, signs of acute ischemic damage are highlighted in alterations of proximal tubuli. Proximal tubuli begin to lose their brush border, with accumulation of amorphous debris in the lumen, sometimes also with loss of nuclear material (small black arrow), while cytoplasm is often clarified, vacuolated, and swollen; basement membrane begins to become thicker around some tubuli. These alterations can progress to complete tubular necrosis, but an important pitfall is that they can represent a fixation artifact when they are suspiciously widespread to the whole biopsy. PAS, 20×magnification
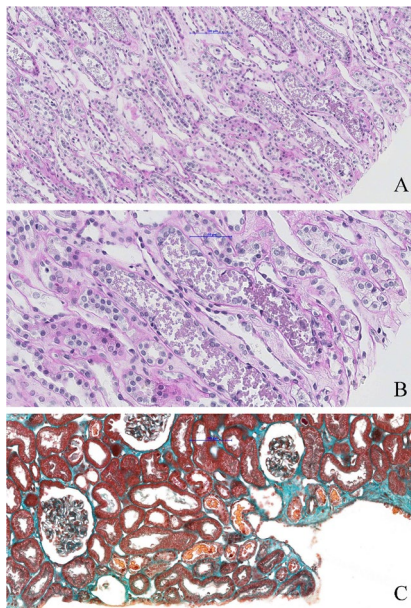
Fig. 7 A–C At 20×magnification, the tubules of this young donor dead in an accident show the presence of globular eosinophilic material in the lumen. It can resemble red blood cells, but at higher magnification (**B**), its appearance is not dirty and dusty as the debris in tubular necrosis, but the globules appear more compact and clearly defined, while with trichrome stain (**C**), they emerge clearly in orange/red as globular material in the tubular lumens. **A** PAS, 20×magnification. **B** PAS, 40×magnification. **C** Trichrome, 20×magnification
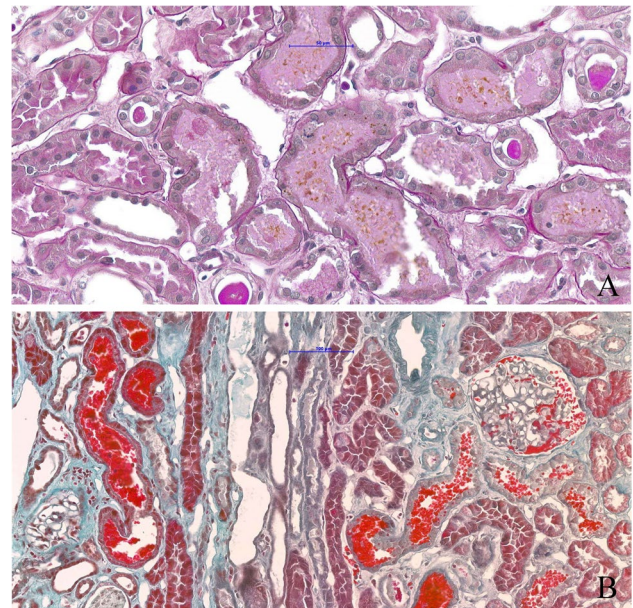

Fig. 8 A–B In this 40×magnification, we have another type of tubular intraluminal material accumulation. The material is mixed pink/orange-brown in PAS, and at high magnification, an impression of "packed globules" can be appreciated, and the tubuli also show signs of acute damage with crushing of cytoplasm and tempted regeneration with mitosis. The trichrome stain (**B**) reveals the nature of the material: they are red blood cells tightly packed in tubuli, and they occupy also the urinary space in glomeruli. The biopsy comes from a young donor with piastrinopenia and subarachnoid hemorrhage, this alteration is due to the preexistent piastrinopenia, and it can be found also in patients taking anti-vitamin K anticoagulants. **A** PAS, 40×magnification. **B** Trichrome, 20×magnification

Signs of Chronic Damage (Figs. 9, 10, 11, 12, 13, 14, and 15)

Fig. 9 This close view of an artery shows not only as lamination of internal lamina elastica and a reduced lumen, thus implying a score 3, but also the presence of cholesterinic emboli in its wall. This finding is not common, but it is considered a risk factor for subsequent vascular rejection in the recipient, and often is accompanied by signs of tubular acute damage or necrosis, atrophy (see the thickened basement membranes) and hypoperfusion of glomeruli for microembolism. PAS, 20×magnification
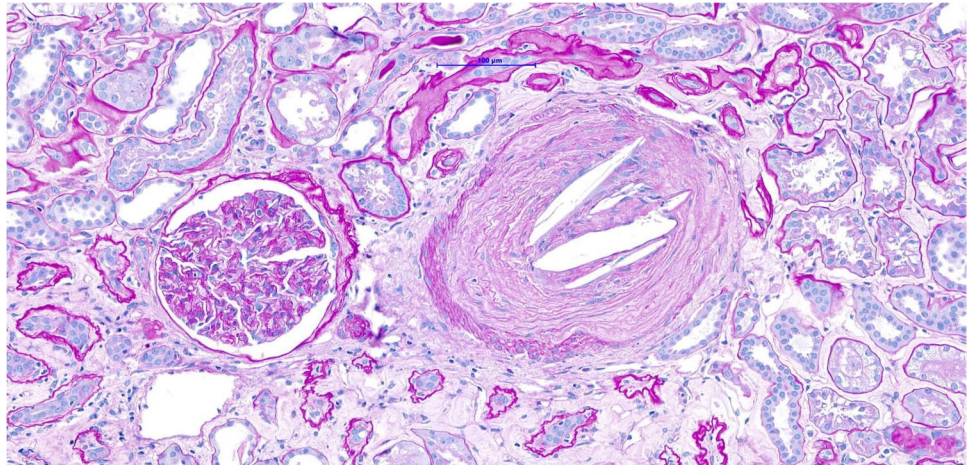
Fig. 10 Another common chronic damage is diabetic glomerular sclerosis. In this 20×magnification, we see two glomeruli where the PAS stain highlights the expansion of the mesangial matrix and the presence of clear hyalinosis of the glomerular arterioles (stars). PAS, 20×magnification
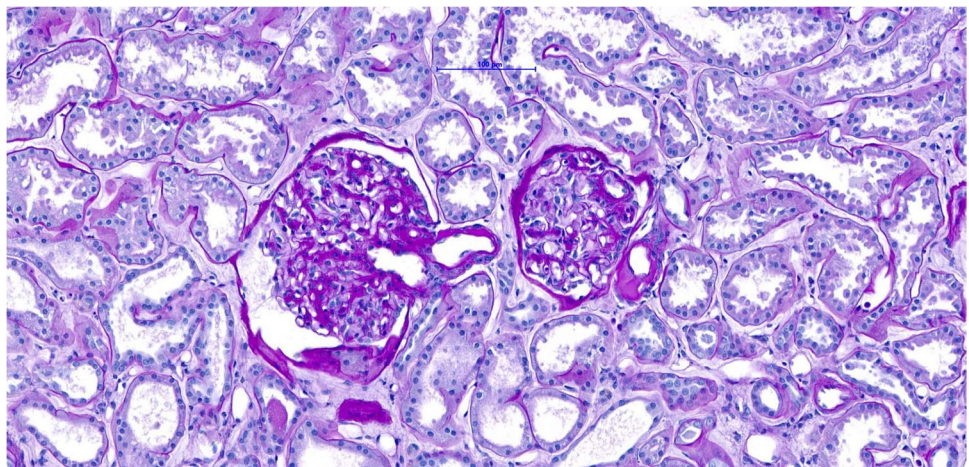

Fig. 11 Another example of mesangial expansion in diabetic damage without nodular sclerosis. The mesangium is broadly expanded without increase of cellularity and without formation of nodular globules and the arterioles show hyalinosis. PAS, 20×magnification
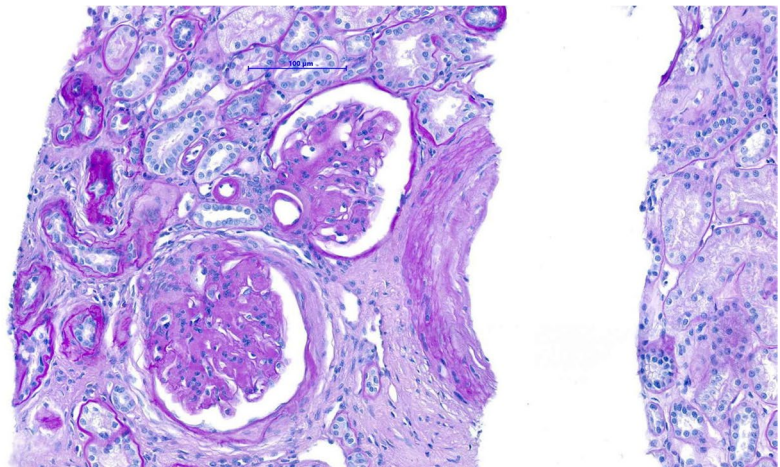
Fig. 12 In this 40× magnification, the mesangial expansion in diabetic damage is even greater and begins to have vaguely nodular appearance, with the characteristic color quality of basement membranes with PAS stain. Note also the arteriole with hyalinosis between the two damaged glomeruli. PAS, 40× magnification
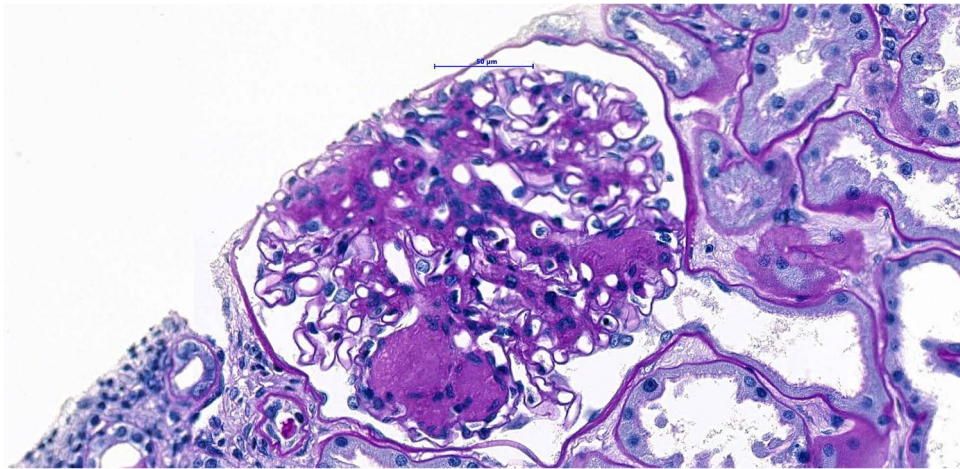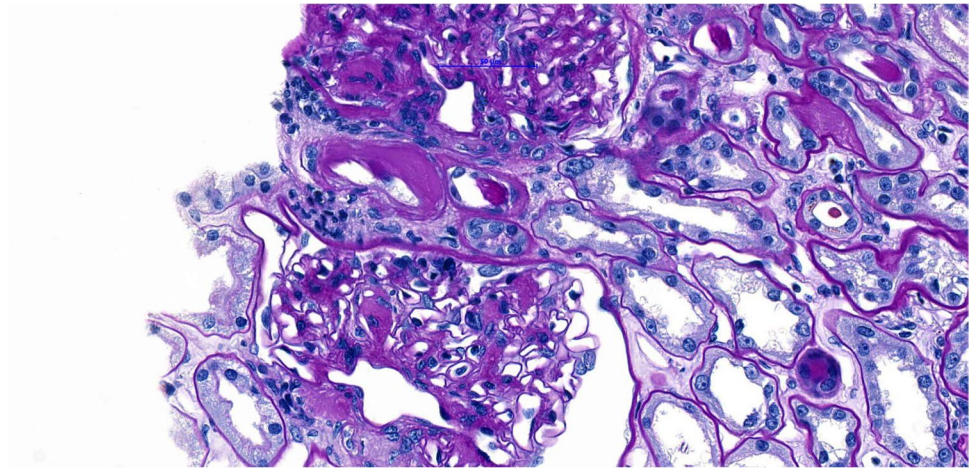

Fig. 13 In the same case, the diabetic damage has progressed to the formation of the typical Kimmstiel-Wilson nodules, globular of intensely PAS-positive material with nuclei at the external border disrupting the normal architecture of the glomerulus, which shows also dilated capillaries. This is a sign of severe and advanced diabetic damage which is important to recognize in the transplant biopsy for its potential influence on graft outcome, while not nodular and mild mesangial expansion have less influence. PAS, 40× magnification
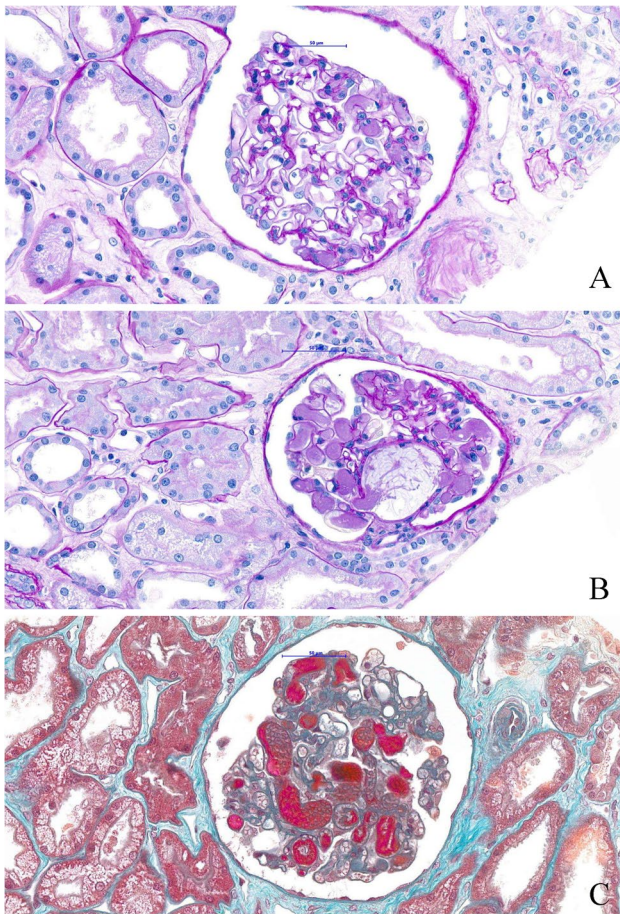
Fig. 14 **A–C** Another common alteration which is important to recognize is thrombotic microangiopathy (TMA): this alteration is classically described in donors after cardiac death (DCD), but it can be encountered also in the more common brain-dead donors (DBD). In these 40×magnifications, the glomerular capillaries are filled for less of 25% (**A**) and for more than 50% (**B**) by fibrinoid material. Note also the coexistent acute tubular damage, with dirty-like detached intraluminal material in some tubules and loss of cellular detail. The trichrome stain (**C**) highlights the packed red blood cells with fibrin in the glomerular capillaries. **A** and **B** PAS, 40×magnification; **C** trichrome, 40×magnification

Fig. 15 In this other example at higher magnification from a biopsy of a DBD donor with trichrome stain, the packed red blood cells with fibrin are clearly visible, and also the acute tubular damage is appreciable with the detached material in the tubular lumens and the debridement of tubular structures. Trichrome, 40×magnification
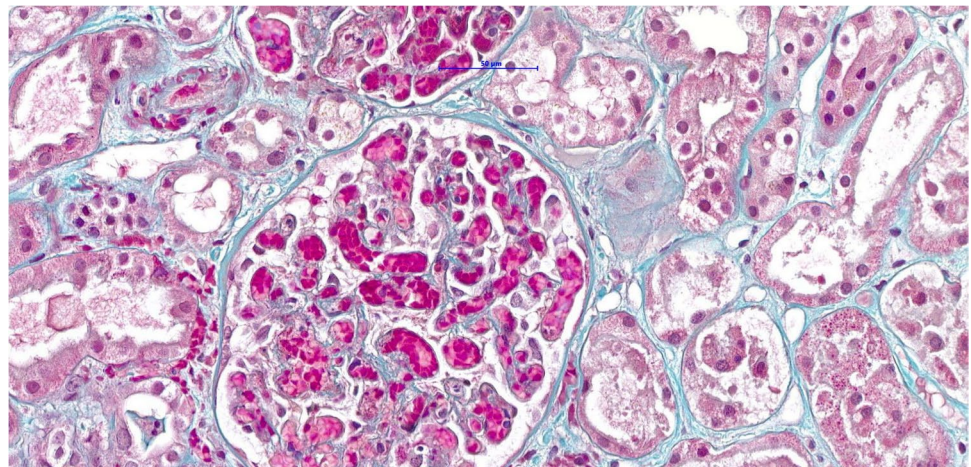
Pearls and Pitfalls (Figs. 16, 17, 18, and 19)

Fig. 16 In this biopsy, the main findings are represented by a diffuse acute inflammatory infiltrate and the presence of numerous fungal spores (circle). The biopsy came from a donor who had undergone extracorporeal membrane oxygenation for another previous transplant, and fungal sepsis is one of the most common complications of this procedure. PAS, 40×magnification
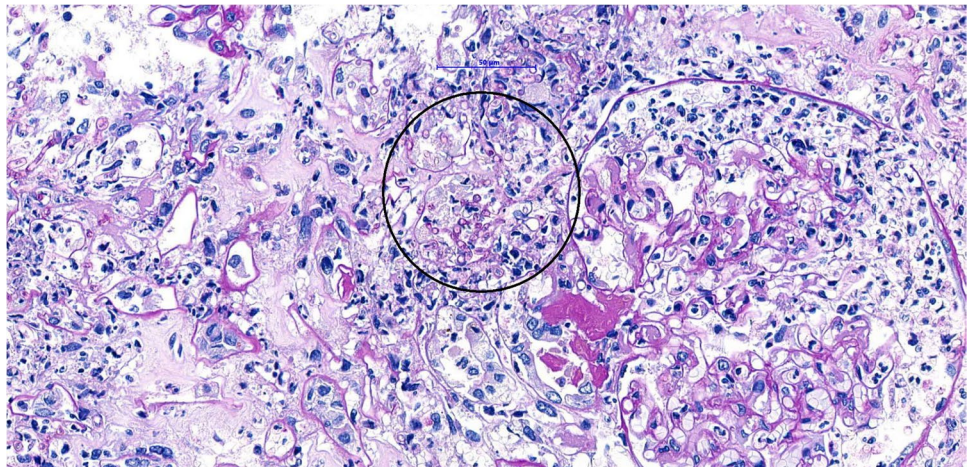

Fig. 17 This biopsy came from a donor of African descent with hypertension but without other clinical information. The small arteries in this field show myxoid degeneration of the subendothelial arterial wall which represents the clue for the disease known as nephrosclerosis maligna. These alterations are considered as a form of thrombotic microangiopathy. PAS, 40×magnification
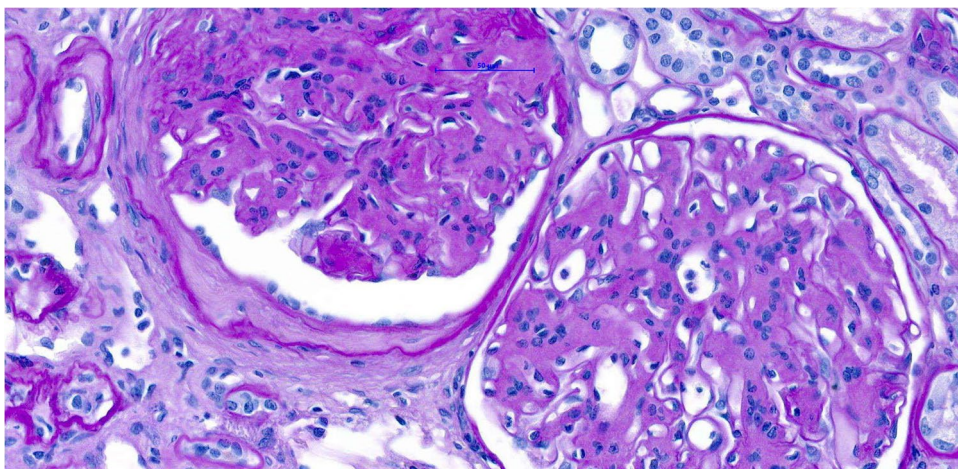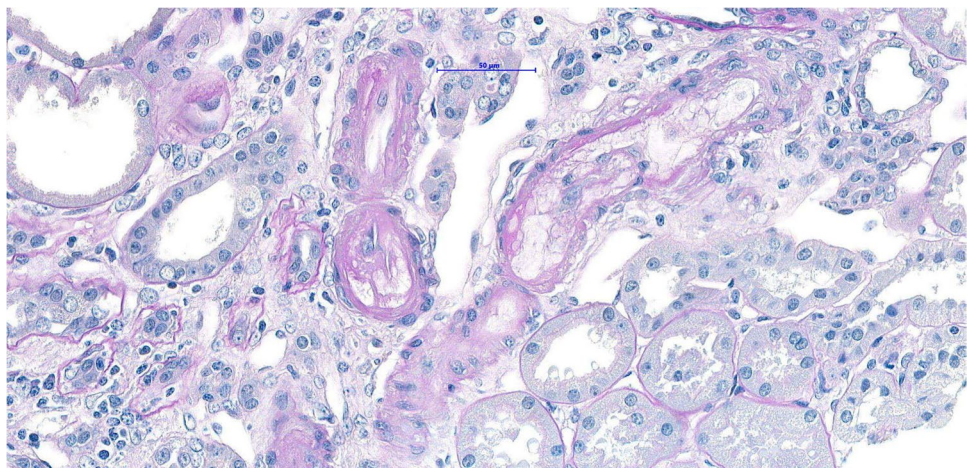

Fig. 18 In this close view of two glomeruli, the main alterations are mesangial expansion and hypercellularity, similar to what found in diabetic not nodular damage. However, the staining quality of the matrix with PAS is a little paler than what found in diabetes and shows a vaguely fibrillary appearance. In other glomeruli of the biopsy, there are similar alterations, and nodular formations are never encountered. These modifications are suspicious of fibrillary glomerulonephritis, a rare disease which is usually diagnosed with ultrastructural analysis and which can be rarely found sporadically in donors. PAS, 40×magnification

Fig. 19 The glomerulus in the box shows the alterations of focal segmental glomerular sclerosis (FSGS) which is usually found in conjunction with tubular atrophy and interstitial fibrosis (see the parenchyma outside the box). Moreover, glomeruli are usually hypertrophic, and an easy rule of thumb to decide on the size of the glomerulus is to consider the radius of the 40× ocular magnification: if a glomerulus is larger of this size, it can be considered hypertrophic. PAS, 40× magnification
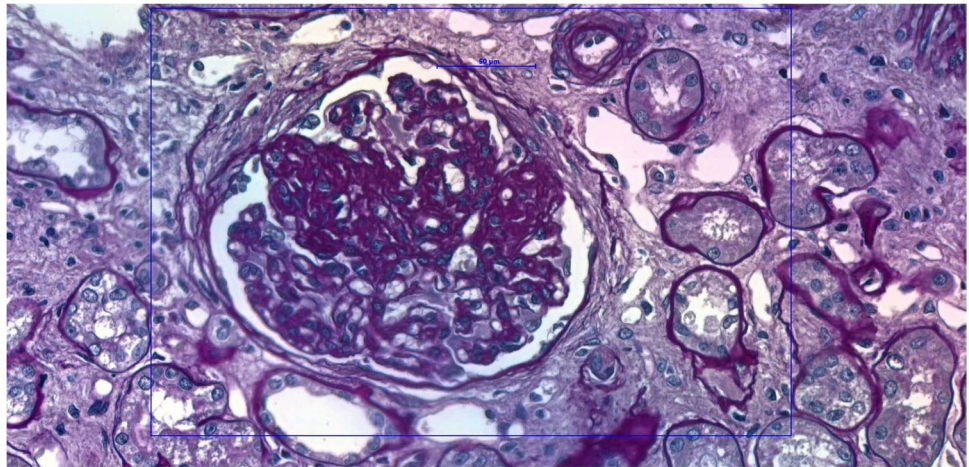
Final Remarks

Simulation-based learning via digital pathology provides learners with a real-world-like opportunity to develop and practice their knowledge and skills but in a simulated environment. Digital events like this highlight the positive potential of digital solution for delivering knowledge to the interested audience even for niche fields of pathology like transplant pathology and offer the opportunity to improve the interaction of the several actors (clinicians, diagnosticians, technical professionals, vendors etc.) of a healthcare community.

Declarations

Human and Animal Rights Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Competing Interests The authors declare no competing interests.

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● Of importance
●● Of major importance

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