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Review

Histology for nephrology, from pre-implantation to post-transplant kidney biopsy. Lesson learned from ReBIrth (Renal Biopsy for Kidney Transplantation Therapy)

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Summary

A meeting entitled Renal Biopsy for Kidney Transplantation Therapy (ReBIrth) took place on May 31st, 2022 in Bologna, Italy. The meeting drew together nephrologists, surgeons, and pathologists and recognized as experts in the field of kidney transplantation in Italy. In this paper, we present our experience working with kidney transplants in the current era of immunosuppression therapy. The primary aim is to report the histopathological characteristics of failed kidney allografts after a consensus of experts reviewed the cases on a wholeslide imaging digital platform. Regardless of the cases discussed, digital pathology was reliable in identifying all the morphological and immunohistochemical features required to improve the correct use of immunosuppressive therapy to prevent graft failure and optimize patient management.

Key words: digital pathology, immunosuppression, kidney biopsy, whole-slide imaging, kidney transplantation

Introduction

The implementation of digital pathology into clinical practice is well established, and the continual refinement of the tool is playing a crucial role in the learning approach and the collaborative sharing of knowledge [1]. The community of pathologists is making a great effort to adopt and validate digital technologies in several pathological fields, including organ transplantation [2]. In this scenario, kidney transplant biopsy is the perfect field in which digital pathology can improve clinical management, modulating the therapy of these patients, given the change and expansion of classification and the need for a different expertise.
The essential role of kidney biopsy is undeniable. Along with the collection of clinical/laboratory data, histopathology is crucial in the overall assessment of the kidney's condition for a more precise prognostic evaluation of long-term graft function. However, there is wide variability in biopsy scoring among pathologists. Moreover, the difficult interpretation and ambiguous thresholds used to assess all criteria included in the Banff classification is another important issue with relevant clinical implications [3]. Finally, more than in other areas, multidisciplinary discussion is mandatory for an appropriate clinic-pathological diagnosis.
For all of these reasons, a meeting entitled Renal BIopsy for Kidney Transplantation Therapy (ReBIrth) was organized by Novartis Farma on May 31st, 2022 in Bologna, Italy, which included traditional lectures and interactive sessions in which clinical cases on digital platforms based on wholeslide imaging (WSI) were discussed. The latter session comprised the main part of the meeting. The aim was to promote the exchange of knowledge on kidney transplantation among nephrologists and pathologists by recognized opinion leaders in the field.

Meeting overview

The meeting involved pathologists, nephrologists, and surgeons from all the Italian kidney transplant centers who shared their expertise by reviewing cases using digital technologies for educational and diagnostic purposes to improve the prospects for managing kidney transplantation. Attendees were 31 nephrologists from all over Italy. The event was structured with lectures and interactive multidisciplinary practical sessions involving case discussions. Cases consisted of 48 slides previously digitized with a Panoramic p1000 scanner at 40x magnification and then uploaded on a dedicated web platform. The file sizes of the digital slides ranged from 200 megabytes to 1.5 gigabytes.

Figure 1. Attendees gathered into different working groups during the meeting.

The experts were gathered into four small groups, and the scanned cases were collegially discussed after the slides were reviewed (Fig. 1). This allowed the participants to make multidisciplinary decisions for the management of transplanted patients. The fields discussed were: preimplant diagnosis, acute rejection, chronic rejection and its management, and infectious and neoplastic complications.
For completeness, in the following sections, two cases are reported as examples.

Case 1: Preimplant diagnosis

A former smoker 47-year-old man died from cardiac arrest after a car accident. Bioptic samples of the left and right kidneys were obtained. Neither glomerulosclerosis nor fibrosis nor tubular atrophy was detected. However, a slightly increased thickness of the arteriolar walls was reported. The Karpinski histologic score was 1 in both renal biopsy samples. Nevertheless, intraluminal fibrin occlusive thrombi and intramural fibrin in arterioles were observed in more than 90% of the glomeruli detected (Fig. 2). Therefore, a final diagnosis of thrombotic microangiopathy was rendered.

Case 2: Rejection diagnosis

A 59-year-old woman was diagnosed in 1985 with pauci-immune crescentic glomerulonephritis, which slowly led to chronic kidney failure. In 2011, the patient developed a diffuse large B cell lymphoma (DLBCL), with cervical, lung, and liver involvement, which achieved complete response following chemotherapy. In 2013, the patient started hemodialysis, and six

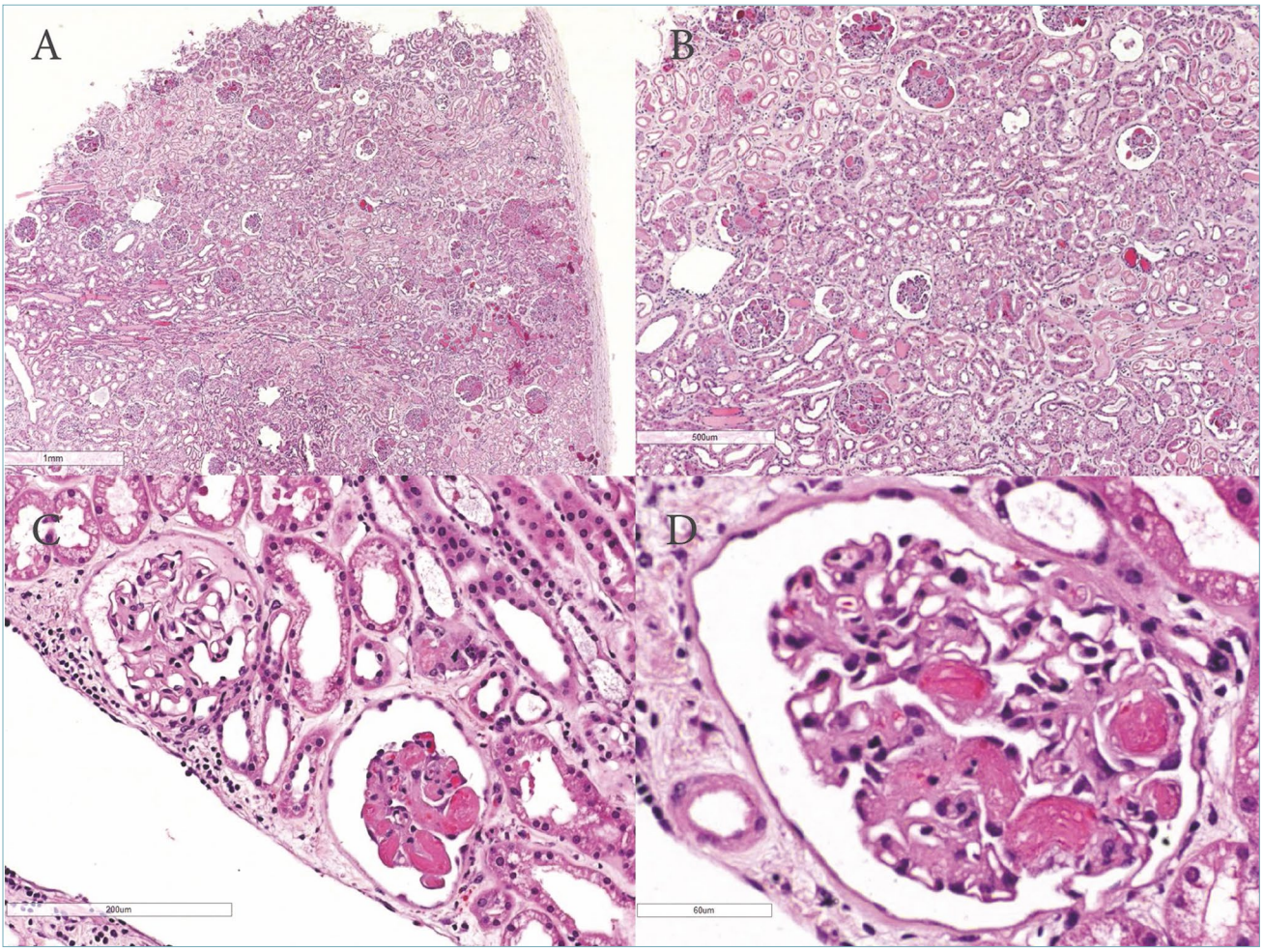

Figure 2. Thrombotic microangiopathy. Low (A) and intermediate (B) magnification of kidney biopsy stained with hematoxylin and eosin showed thrombotic microangiopathy in several glomeruli. Higher magnification highlights the comparison of a glomerulus affected and a normal glomerulus (C). Intraluminal fibrin occlusive thrombi in the glomerulus are easily observed (D).

months later was referred to the hospital for dilatative cardiomyopathy correlated to previously administered chemotherapy. Furthermore, between 2015 and 2017, the patient suffered from multiple pneumonia episodes.

In 2018, she received a kidney allograft from a deceased donor (55 years old, histologic score 0), after a cold ischemia time of 13 hours and 34 minutes, with a panel reactive antibody value (v-PRA) of 0%. She was given immunosuppressive induction based on basiliximab, tacrolimus, mycophenolate mofetil (MMF), and steroids, with further maintenance therapy relying on tacrolimus, MMF, and steroids. Twenty-three months after transplantation, the patient had a celiac nodal recurrence of DLBCL that was treated with rituximab (4+4). The tacrolimus levels and steroids were then reduced, getting a stable renal function (serum creatinine 1.1-1.5 mg/dL). Donor-specific antibodies (DSA) had always been negative.

Three months later, the patient was admitted to the hospital for oligoanuria and dyspnea. Her laboratory examinations revealed significantly increased serum creatinine levels (7 mg/dL) with a negative COVID test. A renal biopsy was performed, which demonstrated acute T cell-mediated rejection (TCMR) with severe inflammation in the non-scarred cortex (i3) and a marked tubulitis (t3) with multiple foci of tubular destruction and associated regenerative nuclear irregularities (Figs. 3A, B - PAS staining). An immunohistochemical assay for the BK virus tested negative. Moreover, four arteries showed reactive endothelial changes and endarteritis, associated with fibrinoid necrosis of the wall in three of the vessels (Figs. 3C, PAS; 3D, AFOG staining; 3E, Trichrome staining), which was then scored v3*, because

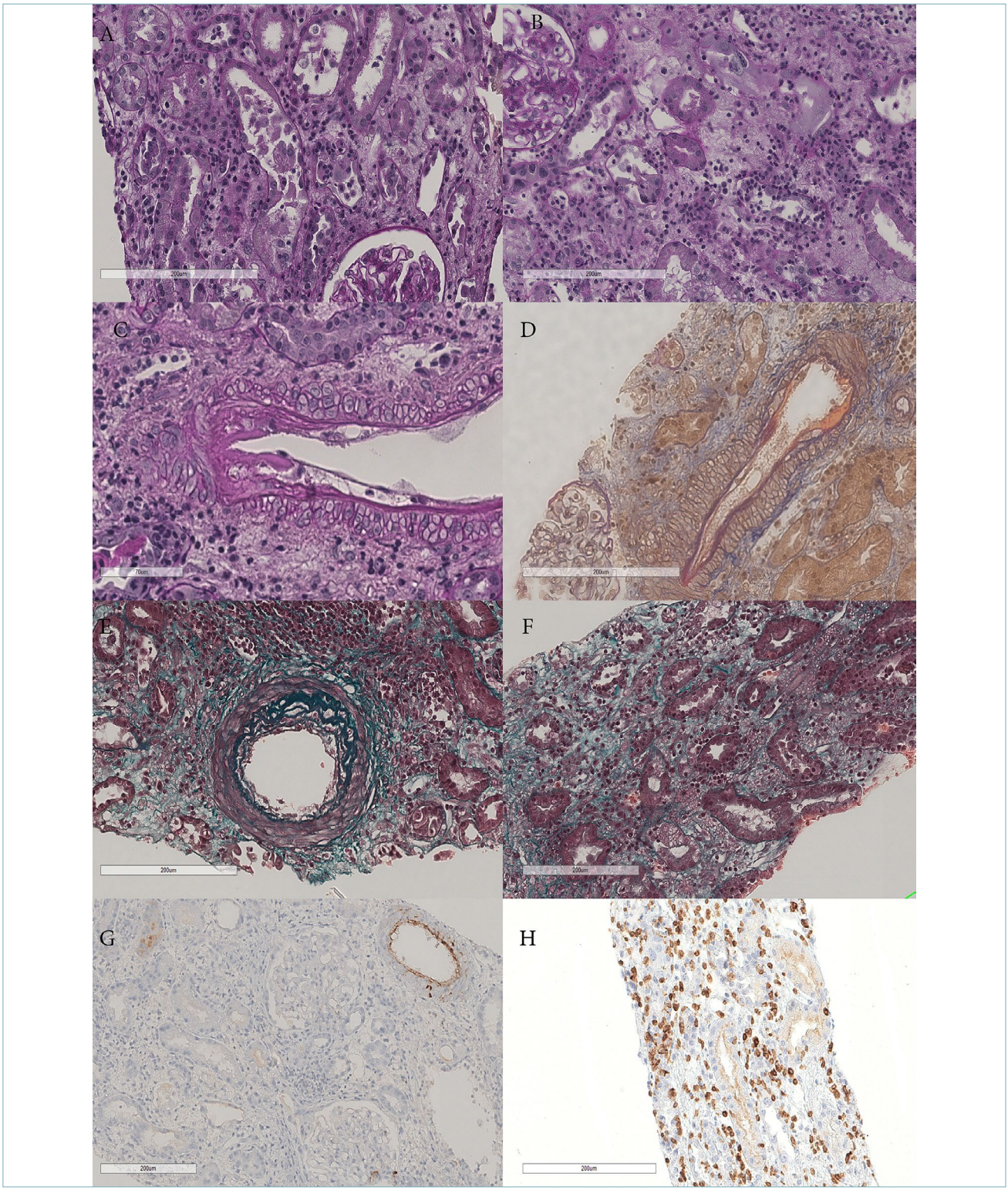

Figure 3. Acute T cell-mediated rejection. Tubulitis and foci of tubular destruction with associated regenerative nuclear irregularities can be seen (A, B: PAS staining). Fibrinoid necrosis of the wall vessels is revealed by using different staining (C: PAS; D: AFOG; E: Trichrome), as well as interstitial hemorrhage (F: Trichrome). C4d immunohistochemistry tested negative (G). The inflammatory infiltrate was made up of small CD3-positive T lymphocytes (H). AFOG: Acid Fuchsin Orange G; PAS: Periodic acid-Schiff.

of the presence of interstitial hemorrhage (Fig. 3F, Trichrome staining). Immunohistochemistry on paraffin-embedded tissue failed to demonstrate C4d deposits (Fig. 3G). The inflammatory infiltrate was made up of small CD3-positive T lymphocytes (Fig. 3H), ruling out the hypothesis of renal involvement by the known lymphoma. Glomeruli were normal, and immunofluorescence on frozen material was negative.
Thus, a final diagnosis of acute TCMR grade III sec. Banff 2019 was made, and the patient began pulse steroid therapy and intravenous immunoglobulin (IVIg).

Discussion

The restricted availability of donor organs compared to the number of patients with end-stage renal disease has led to the employment of several strategies to expand the pool of potential donors and find the best strategy for saving allografts. Histological evaluation of kidney biopsies, along with specific clinical/laboratory data, is one of the main systems routinely employed to guide the proper management of grafts, in both the pre-and post-transplantation settings. Pathological assessment of transplanted kidney biopsies often relies on the recognition of subtle pathological changes, and it has been shown that the evaluation of grafts by experienced nephropathologists may contribute to reducing the number of organs wrongly discarded [4]. However, dedicated transplantation physicians are not available in all institutions, which can potentially affect the overall clinical management of patients. In this scenario, digital pathology can significantly help speed up the standardization of pathological reporting of kidney biopsies. Apart from the physicians' expertise, nowadays, this goal is further hampered by the subjectivity of evaluation and the complexity of the referral scoring systems.
Although the use of digital pathology and WSI in the field of transplantation is still limited, several examples of the application of such a disruptive tool have been reported in recent years, both in the pre and post-transplantation settings [5-7]. First, good concordance rates between WSI and conventional light microscopy have been reported by several studies addressing the feasibility of digital pathology in renal transplantation settings [8], showing comparable results with only slight differences between digital and glass slide assessment that did not affect the allocation of the organs [9]. Second, modern technologies have enabled the creation of easily accessible web-based digital platforms for rapid and safe storing of WSIs, allowing rapid and wide sharing of digitized cases [10]. Along with the recent demonstration of the reliability of small portable devices such as tablets to evaluate kidney transplantation biopsies [11], this would be extremely valuable by allowing resource-limited institutions to quickly get second opinions from trusted experts in the field. Furthermore, computers trained by machine-learning algorithms have started to be developed [12]. These are becoming able to recognize specific pathological features in challenging cases, such as counting and classifying sclerotic glomeruli and quantifying the amount of interstitial fibrosis [13,14].
Despite these considerations, multidisciplinary discussion plays a key role in the overall transplant management approach, and, as far as pathologists are concerned, digital pathology provides an innovative tool for the collegial review of difficult cases by experts. The 2022 Bologna meeting efficiently showed that sharing WSI by the different working groups greatly helped pathologists to reach definitive collegial diagnoses. In fact, the interactions between meeting attendees at the same table led to concordant diagnoses highlighting the pathological clues on the WSI. Moreover, the improvement of conventional diagnostic methods with annotation functionalities, such as the possibility of drawing regions of interest and applying rulers and other objective measuring tools, can significantly contribute to reaching a more homogenous and standardized reporting of kidney transplant biopsies. Finally, the feasibility of currently available digital pathology systems suits the adoption of such tools not only during specifically organized meetings and symposia but also in routine daily practice, which is the ultimate goal that will allow patients to benefit from the best possible clinical care.

Conclusions

The meeting successfully brought together recognized experts in transplantation in Italy demonstrating valuable results for the best management of transplanted patients obtained by using a multidisciplinary approach, where clinicians and pathologists together can discuss cases and review the WSI of kidney biopsies. Although the use of WSI to discuss cases is well-known as a useful tool, its routine use still requires improvement, especially in this particular field.

Conflicts of interest

The authors have nothing to disclose.

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Authors' contribution

Conceptualization: AC and AE. Methodology: AC and AE. Formal analysis and investigation: AC and AE. Writing – original draft preparation: AC and SM. Writing – review and editing: AB, MI, SA, MA, RA, LA, DA, NB, RC, CC, GC, CDB, FDI, AG, FG, GM, VP, AlP, AnP, FP, DP, AR, MR, ER, MPS, LT, PT, GV and GZ. Supervision: AE.

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