An Atlas of Renal Transplant Pathology

An Atlas of Renal Transplant Pathology:

A Clinicopathological Archive

Edited by

Renu Mariam Thomas, Kartik Ganesh
and Sunita Simon

Cambridge
Scholars
Publishing

An Atlas of Renal Transplant Pathology: A Clinicopathological Archive

Edited by Renu Mariam Thomas, Kartik Ganesh and Sunita Simon

This book first published 2023

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-9221-9
ISBN (13): 978-1-5275-9221-6

Dedicated to our families for being with us through this exciting journey

And to all students of Renal transplant Medicine

CONTENTS

CONTRIBUTORS

Abi Abraham M, *MD, DM, DNB, FISN, FASN, FRCP*
Director, Nephrology and Chief of Renal Transplant Services
VPS Lakeshore Hospital and Research Centre, Kochi, Kerala

Renu Mariam Thomas, *FRCPath*
Senior Consultant and Lead Renal Pathologist
Department of Pathology
VPS Lakeshore Hospital and Research Centre, Kochi, Kerala

Kartik Ganesh, *MD (Internal Medicine), DM (Nephrology)*
Consultant
Department of Nephrology and Renal Transplantation
VPS Lakeshore Hospital and Research Centre, Kochi, Kerala

Sunita Simon, *DNB (Internal Medicine)*
Junior Consultant
Department of Nephrology and Renal Transplantation
VPS Lakeshore Hospital and Research Centre, Kochi, Kerala

Georgy K. Nainan, *MD, MNAMS, DM, FISN, FRCP*
Senior Consultant
Department of Nephrology and Renal Transplantation
VPS Lakeshore Hospital and Research Centre, Kochi, Kerala

Vilesh Valsalan, *DNB (Nephrology)*
Consultant
Department of Nephrology and Renal Transplantation
Specialist Hospital, Kochi, Kerala

Jose P Paul, *MD, DM, MRCP, SCE (Nephrology)*
Consultant
Department of Nephrology and Renal Transplantation
Lisie Hospital, Kochi, Kerala

Vanesa Bijol, *MD*
Professor
Department of Pathology and Laboratory Medicine
North Shore University Hospital, Donald and Barbara Zucker School of Medicine, Hofstra/Northwell, Manhasset, New York

Vinay Nair, *DO*
Associate Professor
Division of Kidney Diseases and Hypertension, Department of Medicine
Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Great Neck, New York

Gayatri Nair, *MD*
Assistant Professor
Division of Kidney Diseases and Hypertension, Department of Medicine
Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Great Neck, New York

FOREWORD

An Atlas of Renal Transplant Pathology is of great importance to junior pathologists as well as to postgraduates in nephrology. This beautiful atlas is a kaleidoscopic review of renal pathology that residents will have seen in their years of training.

With the Banff schema developing over the last 30 years, early detection of rejections, combined rejections and chronic active T Cell mediated rejections even in suboptimal biopsies, is very much possible today.

Transplantation in sensitized recipients needs more attention nowadays as it is increasingly being done. We do come across active antibody mediated rejection (ABMR), chronic active ABMR and recurrence of ABMR in a second transplant and precipitation of rejection by pyelonephritis in the graft.

Thrombotic microangiopathy (TMA) in renal allograft is being increasingly detected with the advent of advanced renal histopathological studies. TMA can be associated with ABMR, malignant hypertension and calcineurin inhibitor (CNI) toxicity.

One has to be well-versed in infections in renal transplantation and renal histopathology is very useful in detecting polyoma virus nephropathy, CMV nephritis, and graft pyelonephritis.

Recurrence of primary kidney disease in the allograft is increasingly detected as more native kidney biopsies are now available. IgA nephropathy, Henoch-Schoenlein purpura, focal segmental glomerulosclerosis, and membranous glomerulonephritis are all known to produce recurrence in the allograft.

Pre-implantation biopsies in cadaver transplants have increased the donor pool and have helped us in eliminating suboptimal grafts. This is to be increasingly done in every institution where cadaver kidney transplantations are being done. Renal pathology support is of very great importance especially with urgent reporting in the odd hours of the day.

I have great pleasure in writing this foreword for "Atlas of Renal Transplant Pathology" which I am sure will give guidance to budding nephrologists and pathologists.

With best wishes

Dr. Georgy K. Nainan MD, MNAMS, DM, FISN, FRCP
Sr. Consultant Nephrologist
VPS Lakeshore Hospital and Research Centre
Kochi, Kerala, India

PREFACE

Kidney transplantation is the optimal treatment for improving the survival and quality of life of patients with end-stage renal disease (ESRD). Though recent advances in immunosuppressive and monitoring protocols have led to a significant improvement in the overall allograft outcomes, both acute as well as chronic rejections continue to affect the allograft function and survival. Renal allograft biopsy is the most important tool and gold standard for the evaluation of graft dysfunction. Allograft biopsy is performed to detect the cause of graft dysfunction and the response to treatment and to prognosticate the long-term outcome. In addition, protocol biopsies and baseline donor kidney biopsies are also increasingly being performed. Allograft biopsies often pose a challenge for the pathologist since the allograft can develop all the diseases of the native kidney in addition to the disease specific to the allograft and a quick report is often sought by the clinician.

The Banff classification established in 1991, with its periodic modification is the most accepted grading system for the diagnosis and staging of renal allograft rejection.

This Atlas of Renal Transplant Pathology highlights 30 cases from our day-to-day experience with a brief clinical history, representative photomicrographs with detailed descriptions, the treatment and outcome. These cases have been carefully selected to be of interest to the practicing clinicians, in real-life situations. I am sure this Atlas of Renal Transplant Pathology will serve as a concise practical guide to the interpretation of transplant pathology and will appeal to practicing nephrologists, renal pathologists, renal transplant surgeons and trainees. This book will also encourage them to develop an interest in the understanding of renal transplant related pathology at the microscopic level which will ultimately guide them to plan optimal treatment strategies.

Dr. Abi M Abraham, MD, DM, DNB, FISN, FASN, FRCP
Director, Nephrology and Chief of Renal Transplant Services
VPS Lakeshore Hospital and Research Centre
Kochi, Kerala, India

ACKNOWLEDGEMENTS

"It takes a village" goes the saying, and indeed, the compilation of this atlas would not have been possible without the tremendous support and encouragement we got from our entire transplant team. Kidney transplantation is an intricate, laborious and often vexing process, and we are only able to meet these challenges with the help of a large support system. The compilation of this atlas was a labor of love and would not have been possible without multi-department support. We would like to express our deep gratitude and thanks to our entire team for getting us over the line.

Renu Mariam Thomas, Kartik Ganesh, Sunita Simon

- Dr. Pushpa Mahadevan, Senior Consultant and Director of Laboratory Services, VPS Lakeshore Hospital and Research Centre, Kochi, Kerala, India
- Dr. Georgy K Nainan, Senior Consultant Nephrologist, VPS Lakeshore Hospital and Research Centre, Kochi, Kerala, India
- Dr. Abi M Abraham, Director, Nephrology and Chief of Renal Transplant Services, VPS Lakeshore Hospital and Research Centre, Kochi, Kerala, India
- Dr. George P Abraham, Senior Consultant, Urology and Renal Transplantation, VPS Lakeshore Hospital and Research Centre, Kochi, Kerala, India
- Dr. Datson George P, Consultant Urologist, VPS Lakeshore Hospital and Research Centre, Kochi, Kerala, India
- Dr. Jithin S Kumar, Consultant Nephrologist, Department of Nephrology and Renal Transplantation, VPS Lakeshore Hospital and Research Centre, Kochi, Kerala, India
- Dr. Alok Sharma, Technical Director, Renal Pathology and Transmission Electron Microscopy, Dr LalPathLabs Ltd, New Delhi, India
- Dr. Thara Pratap, Senior Consultant Radiologist, VPS Lakeshore Hospital and Research Centre, Kochi, Kerala, India
- Dr. Ami Maria Emmanuel, Consultant Pathologist, VPS Lakeshore Hospital and Research Centre, Kochi, Kerala, India

- Dr. Tena Thomas Abraham, Department of Nephrology and Renal Transplantation, VPS Lakeshore Hospital and Research Centre, Kochi, Kerala, India
- Dr. Sheenu Sara Varghese and Dr. Dhanya PG, Department of Pathology, VPS Lakeshore Hospital and Research Centre, Kochi, Kerala, India
- Dr. Avantika Gaur
- Mini Jose, Smruthi Prabhat, Princy Antony, Bibi KM and Francina Manuel for technical support.

CHAPTER 1

INTRODUCTION

RENU MARIAM THOMAS

Kidney transplantation is the treatment of choice in patients with end-stage renal disease as it offers the best quality of life. Renal allograft biopsy, until this day, remains the gold standard for evaluation of allograft dysfunction. It is the most powerful tool to provide information with therapeutic and prognostic implications.

Indications for allograft biopsy

- Primary graft non-function
- Slow graft function
- Delayed graft function
- Acute deterioration in graft function – which can occur at any time in the post-transplant period.

Types of transplant biopsies

- **Donor biopsy** to evaluate the quality of the donor kidney in cadaveric transplants especially in expanded criteria donors
- **Zero-hour biopsy** taken immediately after kidney implantation that provides the baseline status of the implanted kidney
- **Protocol biopsy (surveillance biopsy)** taken at specific time points in patients with stable allograft functions – there are no universal guidelines regarding the numbers and time points for these biopsies and it is entirely at the discretion of each transplant center. It provides a baseline for post-transplant pathology and provides information to improve graft outcome such as detection of subclinical rejection and early chronic rejection, early diagnosis of recurrent disease and evidence for under or over immunosuppression.
- **Diagnostic (Indication) biopsy**

Adequacy

- Biopsy cylinders with a minimal length of 1 cm and a diameter of at least 1.2 mm with a minimum of 10 glomeruli and 2 arteries are recommended as optimal for transplant biopsy work up.[1] It is recommended that at least two separate cores containing cortex are obtained or that there are two separate areas of cortex in the same core. One core is to be immediately transferred to 10% neutral buffered formalin for light microscopy. The other core for immunofluorescence microscopic studies is immersed in a transport medium and transferred to the renal pathology laboratory.
  >Do not crush >Avoid forceps (use wooden stick) >Do not allow to dry out
- Both containers have to be properly labelled with the patient`s identification details and the fixative/ transport media.
- A request form with the patient`s identification details and all relevant clinical and laboratory findings should accompany the biopsy – type of transplant (living donor or cadaveric), native kidney disease, time post-transplant, immunosuppression details, baseline and current serum creatinine levels, level of proteinuria, drug trough levels, and co-morbidities like diabetes/hypertension.

Microscopic diagnosis is a subjective evaluation that acquires full meaning only when the pathologist is fully cognizant of the essential clinical and laboratory data.

Reference

1. Racusen LC, Solez K, Colvin RB, Bonsib SM, et al. The Banff 97 working classification of renal allograft pathology. Kidney Int. 1999 Feb; 55(2): 713-23. doi: 10.1046/j.1523-1755.1999.00299.x. PMID: 9987096.

CHAPTER 2

HANDLING OF RENAL ALLOGRAFT BIOPSY

RENU MARIAM THOMAS

Similar to native renal biopsy, renal allograft biopsy requires considerable technical expertise and an experienced renal pathologist for identifying the various subtle transplant-related pathologies. The following specialized technical methods are routinely followed in day-to-day practice.

- Light microscopy (formalin fixed tissue) – If marked urgent, the biopsy is rapidly processed to paraffin sections for same-day reporting; otherwise, it is processed overnight for next-day reporting. 30 serial sections are cut to 3-4-micron thickness on 10 slides; slides 2, 4, 7 and 10 are stained with hematoxylin and eosin stain (H&E), slides 3, 5 and 9 with periodic acid-Schiff (PAS), 6 with Masson trichrome (MT) and 8 with Jones` silver methenamine stains (Fig. 2-a). Unstained spares are kept for staining if needed and further deeper sections are cut as required.

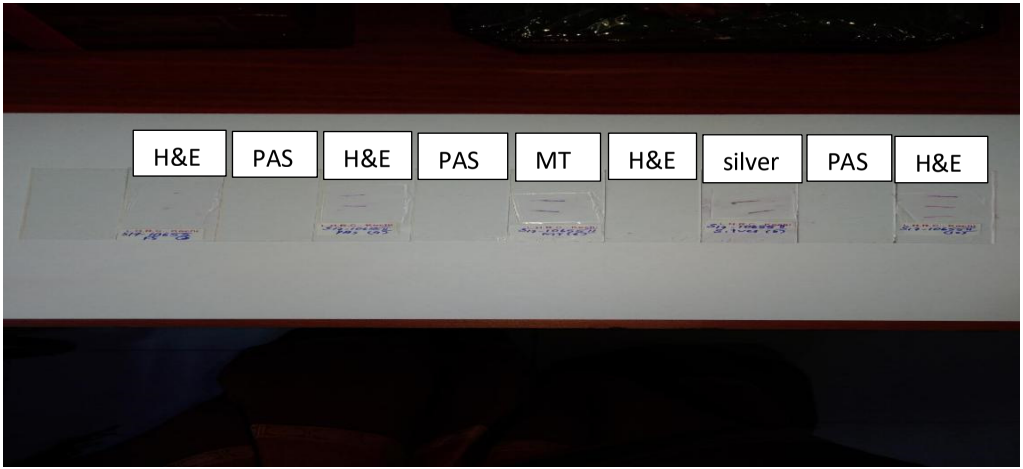

Fig. 2-a. The order in which various stains are used in our laboratory

Hematoxylin and eosin (H&E) is the best general stain for evaluating the different compartments in the biopsy. Periodic acid Schiff (PAS) (Fig. 2-b) and Jones` silver methenamine stains highlight the basement membranes of glomeruli and tubules. Masson trichrome stain (MT) picks up necrosis, fibrosis, thrombi and immune complex deposits (Figs. 2-c, d).

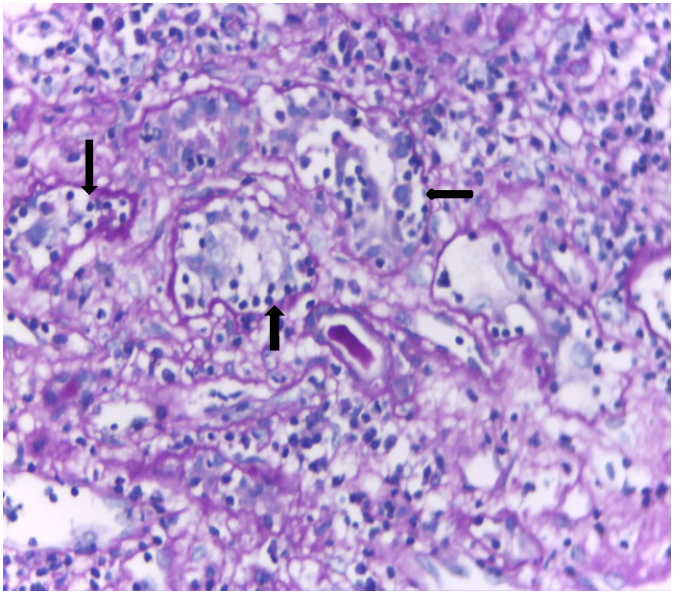

Fig. 2-b. PAS stain highlighting tubular basement membranes and tubulitis. 40x

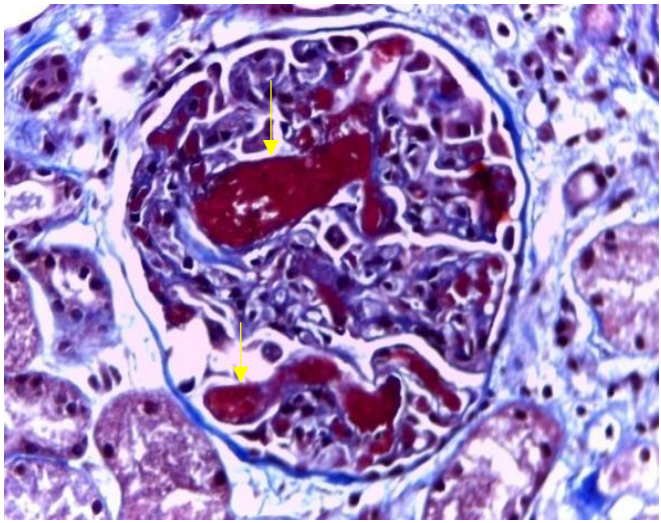

Fig. 2-c. Masson trichrome stain (MT) highlighting glomerular thrombi-arrows. 40x

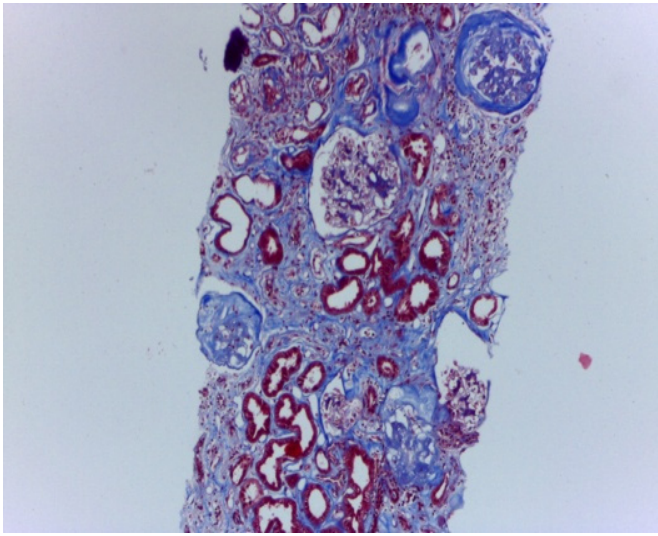

Fig. 2-d. Blue staining of interstitial fibrosis on MT stain helps to assess the extent of chronic parenchymal damage. 40x

- C4d, a complement split product which gets deposited along peritubular capillaries in antibody mediated rejection is done routinely, either using the indirect immunofluorescence method on a fresh cryostat section (Fig. 2-e) or by immunohistochemistry (IHC) using peroxidase on formalin fixed tissue (Fig. 2-f).

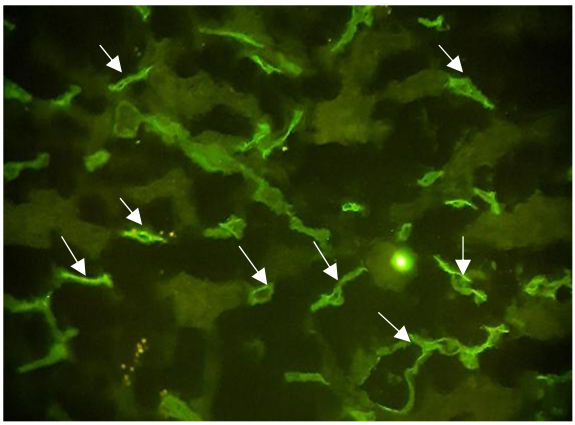

Fig. 2-e. Indirect immunofluorescence staining for C4d (murine monoclonal anti-human C4d, Dako followed by fluorescein isothiocyanate) showing linear staining along peritubular capillaries (arrows). 40x

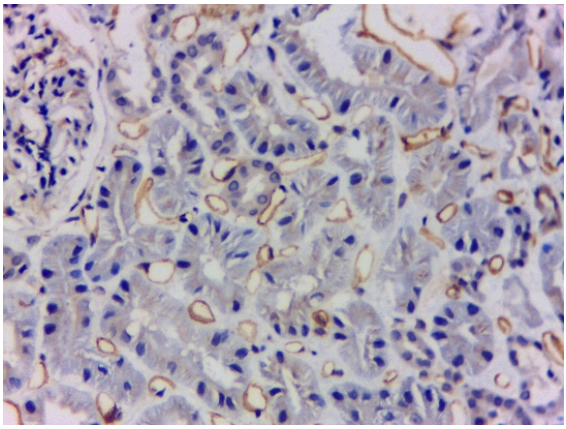

Fig. 2-f. Immunohistochemical (IHC) staining for C4d (rabbit polyclonal antibody, Biogenex) showing brown circumferential linear staining along peritubular capillaries (Bond Polymer refine detection method using Leica BOND MAX fully automated IHC machine). 40x

- Immunoglobulins and complements (on fresh cryostat sections) – optional, depending on the departmental policy to detect recurrent/de novo glomerulonephritis.
- Immunohistochemistry-antibody to SV40T antigen for polyoma virus nephropathy and cytomegalovirus antibody – if indicated.
- Electron microscopic studies – recommended but not mandatory – detect early recurrent/de novo glomerulonephritis and early detection of transplant glomerulopathy.

Banff recommends EM studies in all biopsies >6 months and all indication biopsies >3 months post transplant[2]

Inadequate biopsy

- If the tissue obtained is insufficient, a discussion with the pathologist is necessary to address how best to proceed, before the tissue is placed in fixative so as to obtain the maximum information from the available tissue for a specific clinical scenario.
- In early post-transplant biopsies, if a recurrence of native kidney disease is not considered, light microscopic studies along with C4d immunohistochemistry (IHC) on paraffin processed tissue can provide sufficient information. Immunofluorescence studies can be avoided (see Case 2, chapter 4).
- The tissue core, after taking the necessary cryostat sections for immunofluorescence studies, can be reprocessed to paraffin tissue for light microscopy. This tissue may provide valuable information that may not be present in the main formalin fixed tissue (Fig. 2-g-i).

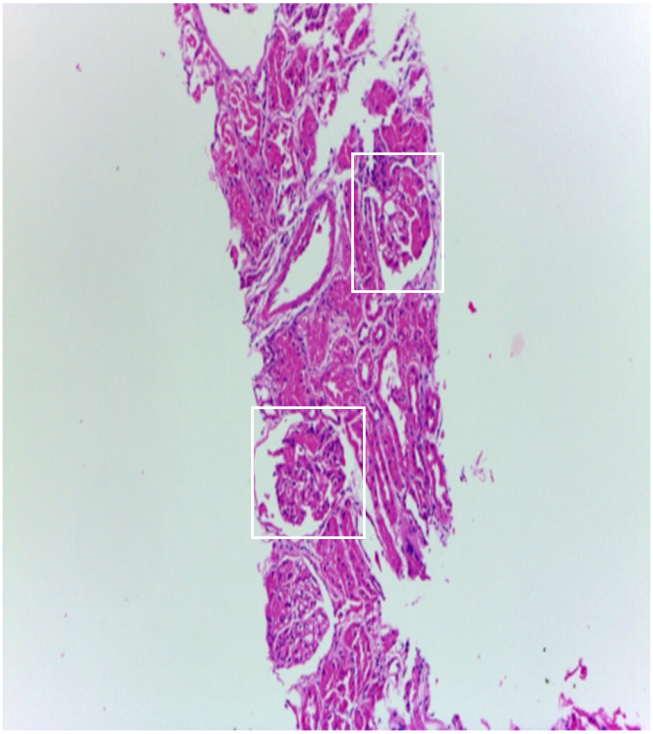

Fig. 2-g. Reprocessed tissue after freezing for IF studies – may reveal significant findings like glomerular thrombi in spite of the freezing artifact. H&E, 10x

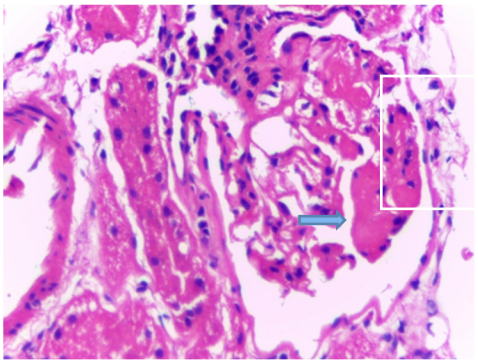

Fig. 2-h. High-power view revealing glomerular thrombi in the reprocessed tissue (arrow). H&E, 40x

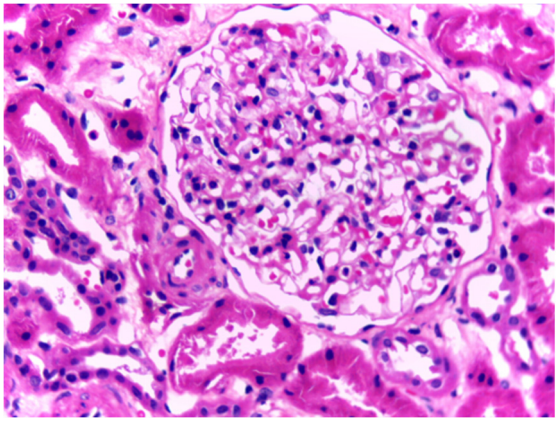

Fig. 2-i. Glomerulus in the formalin fixed tissue (same case) was normal without thrombi. H&E, 40x

Reference

2. Loupy A, Haas M, Roufosse C, Naesens M, et al. The Banff 2019 Kidney Meeting Report (I): Updates on and clarification of criteria for T cell- and antibody-mediated rejection. Am J Transplant 2020 Sep; 20(9): 2318-2331. doi: 10.1111/ajt.15898. Epub 2020 May 28. PMID: 32463180; PMCID: PMC7496245.

CHAPTER 3

EVOLUTION OF THE BANFF SCHEMA (1991-2021)

RENU MARIAM THOMAS

In general, when transplanting tissue from a genetically different donor to the recipient, the alloantigens of the donor induce an immune response in the recipient against the graft. This process is called allograft rejection and it results in inflammation with specific pathologic changes in the allograft, with or without dysfunction of the allograft. Both innate and adaptive immune systems play a significant role in rejection. All compartments of the kidney, i.e., the glomeruli, tubules, interstitium and blood vessels, take part in the rejection process and these changes are scored as per the Banff schema.

Evolution of the Banff Schema (1991-2021)

The Banff classification for renal allograft pathology was first developed by a group of pathologists, nephrologists and transplant surgeons at a meeting in Banff (a resort town in the province of Alberta), Canada on August 2-4, 1991. The goal was to form a framework for the standardized reporting of renal transplant biopsies. It marks a major milestone in the history of transplantation and has continued to evolve through meetings every 2 years and has become the worldwide standard for the interpretation of transplant biopsies.

Following transplantation, donor HLA antigens from the graft are presented to the recipient's T cells by antigen presenting cells, thereby activating CD8+ and CD4+ T cells. CD8+ CTLs may directly destroy graft cells; CD4+ cells secrete cytokines and induce inflammation, which damages the graft. T cells may also react against graft vessels, leading to vascular damage (Fig. 3-a). Based on this understanding, they defined the core pathology lesions as glomerulitis, interstitial inflammation, tubulitis and arteritis. None of these lesions are diagnostic in isolation, hence they set

numerical scores for each of these lesions, based on quantitative thresholds, which were then assembled to form diagnostic categories (Tables 3-1 and 3-2).

There were only 14 participants for the first meeting and it was clear even at that time that this classification needed to be refined as new data emerges. For this reason, they decided to bring the same group together every 2 years and refine the schema. This is now ongoing for 30 years. The last conference at Pittsburgh, PA (USA) was attended by 1253 delegates from 31 countries. It has also expanded into other organs like the liver, lung, heart, etc.

Table 3-1. Numerical codes (Banff 1991[3])

g 0 no glomerulitis
1 glomerulitis in a minority of glomeruli
2 segmental or global glomerulitis in about 25 to 75% of glomeruli
3 mononuclear cells in capillaries of all or nearly all glomeruli with endothelial enlargement and luminal occlusion

i 0 no interstitial inflammation
1 up to 25% of parenchyma inflamed
2 26 to 50% of parenchyma inflamed
3 >50% of parenchyma inflamed

t 0 no mononuclear cells in tubules
1 Foci with 1 to 4 cells/tubular cross section or 10 tubular cells
2 Foci with 5 to 10 cells/tubular cross section
3 Foci with >10 cells/tubular cross section/inflammatory tubular basement membrane break down

v 0 no arteritis
1 Mild-to-moderate intimal arteritis in at least one arterial cross section
2 Moderate-to-severe intimal arteritis in more than one arterial cross section
3 Severe intimal arteritis in many arterial cross sections and/or "transmural" arteritis, fibrinoid change and medial smooth muscle necrosis, often with patchy infarction and interstitial hemorrhage

ah 0 no PAS-positive hyaline thickening
1 Mild-to-moderate PAS-positive hyaline thickening in at least one arteriole
2 Moderate-to-severe PAS-positive hyaline thickening in more than one arteriole
3 Severe PAS-positive hyaline thickening in many arterioles

cg 0, 1, 2, 3 no, mild, moderate, severe chronic transplant glomerulopathy

ci 0, 1, 2, 3 no/up to 5%, mild (6-25%), moderate (26-50%), severe interstitial fibrosis (>50%), often with mononuclear cell inflammation

ct 0, 1, 2, 3 no, mild (up to 25%), moderate (26-50%), severe tubular atrophy and loss (>50%)

cv 0, 1, 2, 3 no, mild, moderate, severe fibrous intimal thickening often with elastica fragmentation (cv3 indicates complete occlusion)

Table 3-2. Diagnostic categories for renal allograft biopsies – Banff 1991[3]

1. Normal

2. Hyperacute rejection

3. Borderline changes ("very mild acute rejection"). This category is used when no intimal arteritis is present, but only mild or moderate focal mononuclear cell infiltration (i1) with the foci of mild tubulitis (1 to 4 mononuclear cells/tubular cross section – t1).

4. Acute rejection

Grade I, mild acute rejection with significant interstitial infiltration (>25% of parenchyma affected – i2/i3) and the foci of moderate tubulitis (>4 mononuclear cells/tubular cross section or a group of 10 tubular cells – t2).
Grade II, moderate acute rejection with (A) significant interstitial infiltration – i2/i3 and the foci of severe tubulitis (>10 mononuclear cells/tubular cross section – t3) and/or (B) mild or moderate intimal arteritis (v1).
Grade III, severe acute rejection with severe intimal arteritis (v2) and/or "transmural" arteritis with fibrinoid change and necrosis of medial smooth muscle cells (v3). Recent focal infarction and interstitial hemorrhage without other obvious cause are also regarded as evidence for Grade III rejection.

5. Chronic allograft nephropathy (Glomerular and vascular lesions help to define the type of chronic nephropathy; new-onset arterial fibrous intimal thickening suggests the presence of chronic rejection.)

Grade I – Mild interstitial fibrosis and tubular atrophy
Grade II – Moderate interstitial fibrosis and tubular atrophy
Grade III – Severe interstitial fibrosis and tubular atrophy and tubular loss

6. Other – changes not considered to be due to rejection

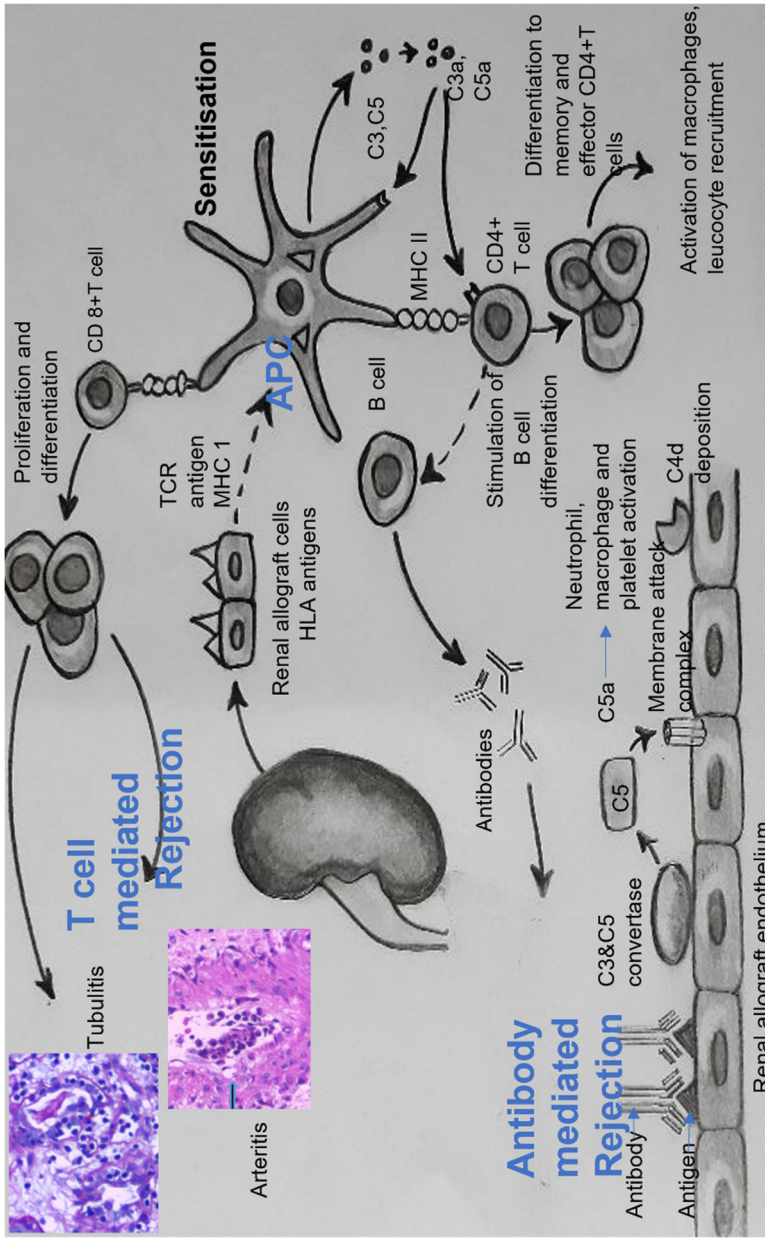

Fig. 3-a. Pathophysiology of organ rejection