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The value of protocol biopsies in renal allografts

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INTRODUCTION

- **Protocol biopsy of an allografted kidney has been introduced in many centers over the world in past years, to determine the presence of acute and chronic lesions in stable, well-functioning allografts.**

- **Biopsy may also detect clinically unsuspected lesions, such as drug induced nephropathy, recurrent original disease, ischemic tubular injury.**

- **The information provided by different centers suggests that acute lesions tend to reach their maximum during the initial months after transplantation, and the incidence of chronic lesions is low during the first month, progressively increasing thereafter.**

Seron D et al: Kidney Int 1997;51: 310-316;
Rush DN et al: Transplantation 1995; 59: 511-514

INTRODUCTION

- **A significant number of cases with acute rejection after kidney transplantation are low-grade forms; they are usually clinically silent but can be recognized at the time of biopsy.**
- **Early diagnosis of CAN as major cause of late renal allograft loss is important to determine treatment strategies.**
- **Protocol biopsies can also provide useful information early in the evaluation process, often before clinical signs of CAN appear and influence clinical management.**
- **Besides that, it allows research on the pathobiology of kidney transplants.**

WHY Banff – CLASSIFICATION (METHODS)

- **We need standardized interpretation of the allograft renal biopsy, this is necessary to ensure adequate treatment and estimation of the end point of transplant rejection.**

Methods included are:

a) analysis of the data in the studies that use Banff classification;

b) using published data acquired in so called collaborative clinical studies for transplantation;

c) international conferences.

Diagnostic categories in Banff classification 1997

Kidney International, Vol.55(1999), pp713-723

1. Normal
2. Hyperacute antibody mediated rejection (immediate and accelerated)
3. Borderline changes (very mild acute rejection): mild to moderate focal mononuclear inflammatory substrate with foci of mild tubulitis (1-4 cells)
4. Acute rejection:
   - Grade 1A- mild acute rejection (>25% of parenchyma affected) + moderate tubulitis (>4 cells / tubular cross section);
   - Grade 1B –significant interstitial infiltration and foci of severe tubulitis (>10 mononuclear cells / tubular cross section)
   - Grade 2A Mild to moderate arteritis (v1)
   - Grade 2B – severe intimal arteritis (v2)
   - Grade 3 – transmural arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells (v3) + lymphocytic inflammation

Diagnostic categories in Banff classification 1997

Kidney International, Vol.55(1999), pp713-723

5. Chronic / sclerosing allograft nephropathy

- **Grade 1 – mild interstitial fibrosis and tubular atrophy with or without specific changes suggesting rejection.**
- **Grade 2 - Moderate interstitial fibrosis and tubular atrophy**
- **Grade 3 – Severe interstitial fibrosis and tubular atrophy and tubular loss**

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

NUMERICAL CODES

- Glomerulitis (G) 0, 1, 2, 3
- Interstitial mononulear infiltration (I) 0, 1, 2, 3
- Tubulitis (T) 0, 1, 2, 3
- Vasculitis (V) 0, 1, 2, 3
- Hyaline arteriolar thickening (AH) 0, 1, 2, 3
- Chronic transplant glomerulopathy (CG) 0, 1, 2, 3
- Interstitial fibrosis with mononuclear inflammation (CI) 0, 1, 2, 3
- Tubular atrophy and loss (CT) 0, 1, 2, 3
- Fibrous intimal thickening and fragmentation of the intima(CV) 0, 1, 2, 3

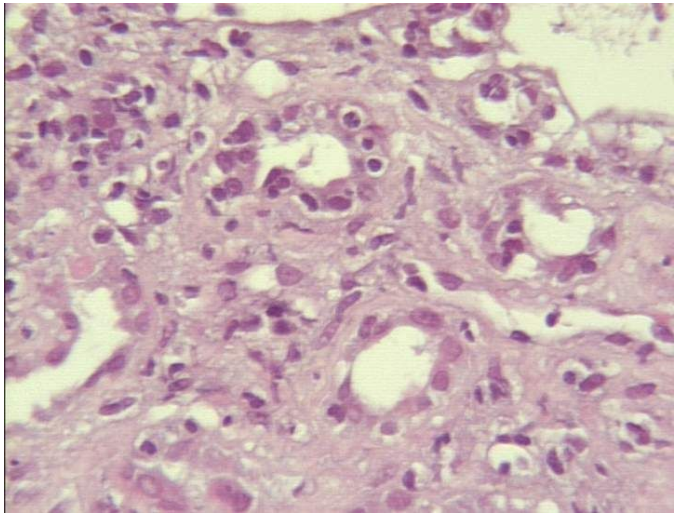
  - Acute and chronic codes are used together
  - Adequacy of the specimen: not satisfied; marginal; adequate.
  - Minimal number of sections: 7 slides with 3 HE, 3 PAS and 1 Trichrome.

Differential diagnosis of other entities

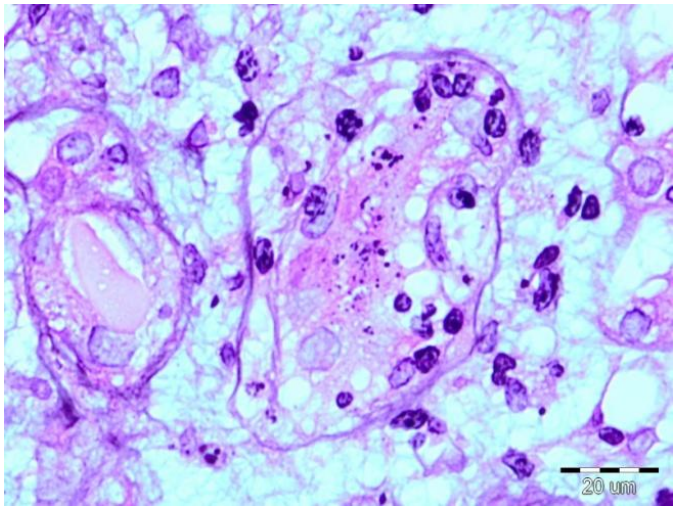
1. Post-transplant lymphoproliferative disorder
2. Nonspecific changes: interstitial infiltration without tubulitis, changes on blood vessels.
3. Acute tubular necrosis
4. Acute interstitial nephritis
5. Changes associated with the application of cyclosporine
6. Subcapsular injury
7. Pre-transplant acute endothelial lesion
8. Papillary necrosis
9. De novo glomerulonephritis
10. Recurrent disease
11. Pre-existing disease
12. Other (viral infection, thromboses, obstruction, lymphocele, urine leak)

Diagnostic categories according to Banff-classification criteria

- Normal findings
- Hyperacute rejection
- Borderline changes (mild tubulitis /1-4 cells, mild interstitial inflammatory cells infiltration)



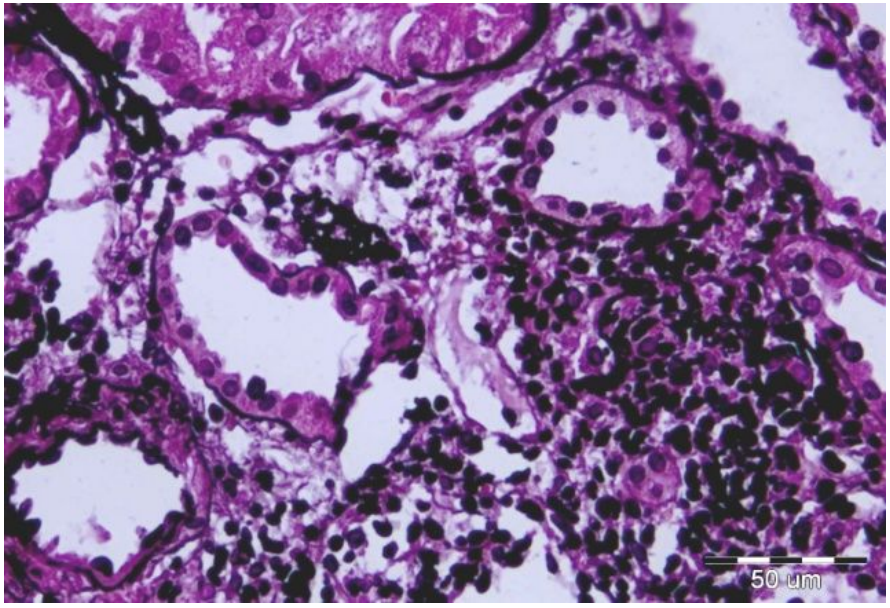
- Acute cellular rejection –tubulointerstitial – grade 1a: (mild interstitial infiltration and focus of mild tubulitis/>4 cells cross tubular section)

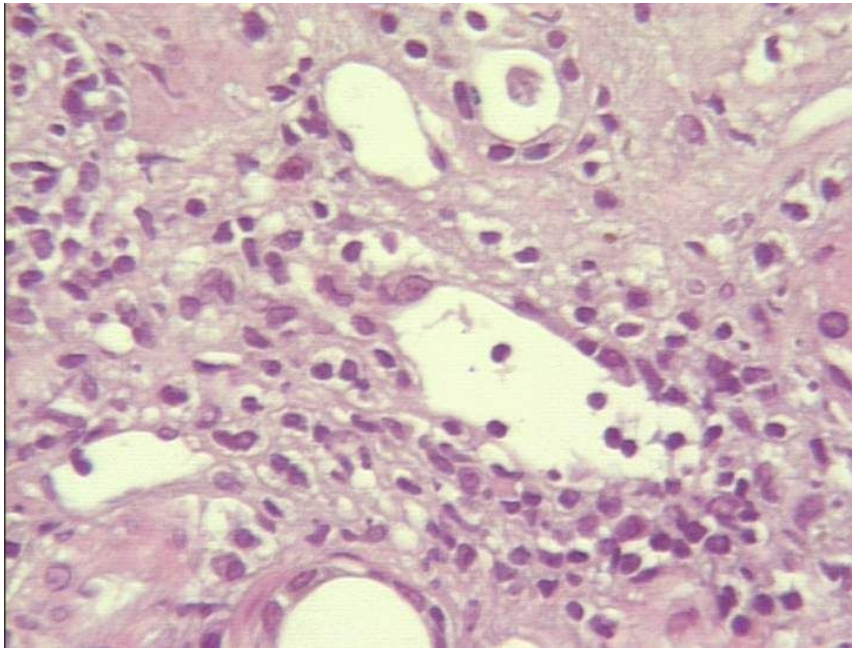


Diagnostic categories according to Banff-classification criteria

- **Acute cellular rejection –**

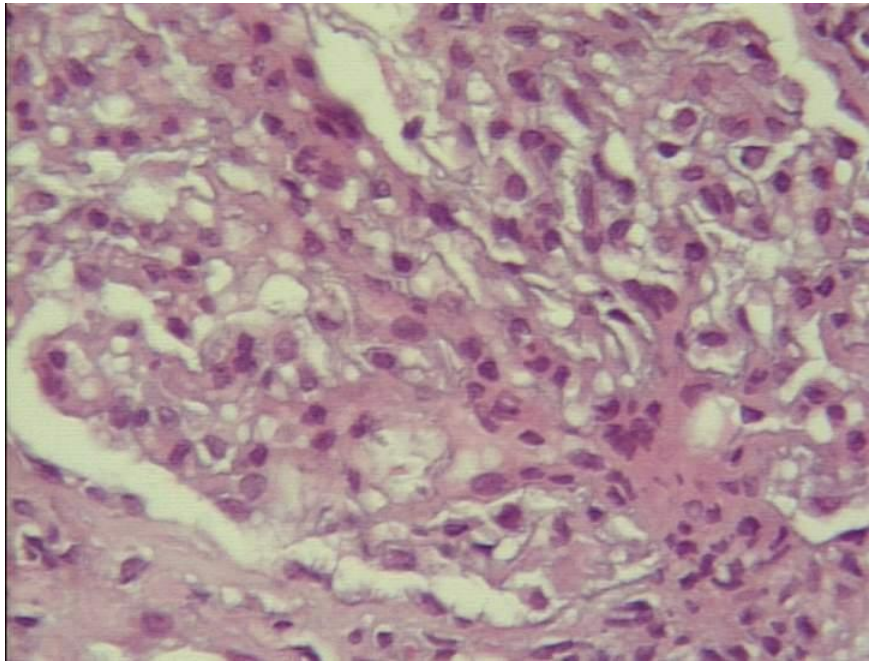
  grade 1b: (moderate to severe interstitial infiltration and focus of severe tubulitis />10 cells cross tubular section/)

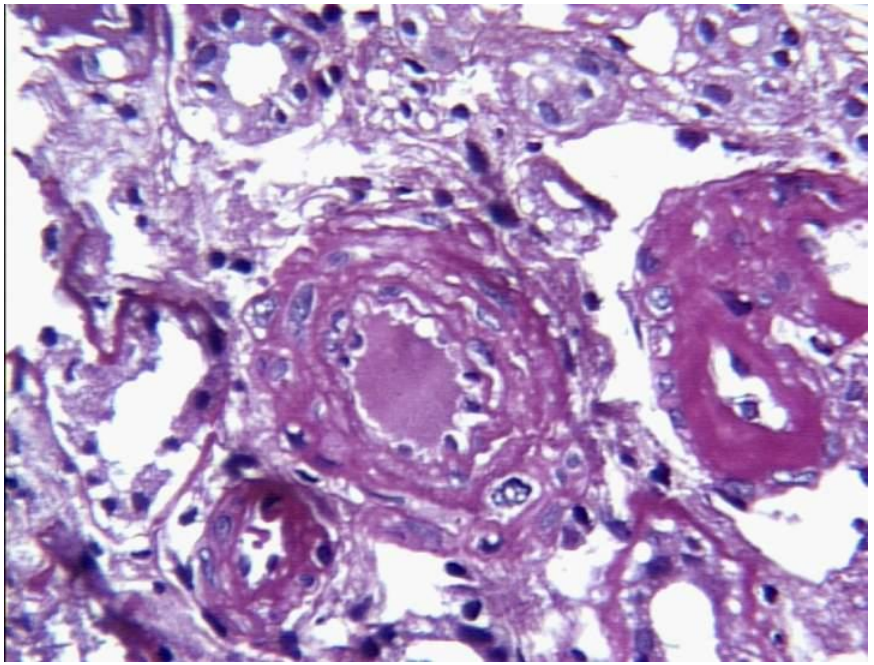




Diagnostic categories according to Banff-classification criteria

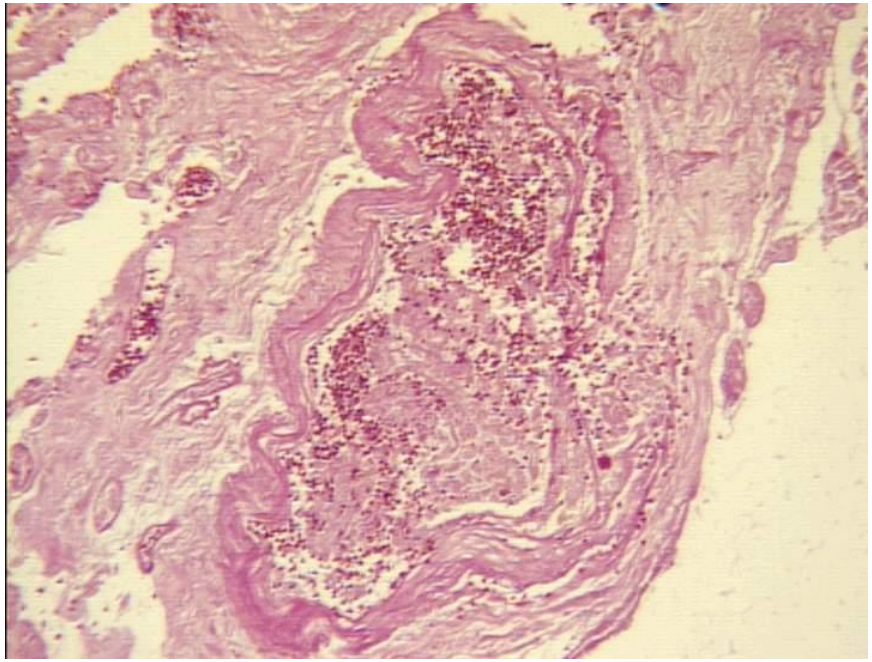
- **Acute vascular rejection (grade 2a/b): Intimal arteritis**

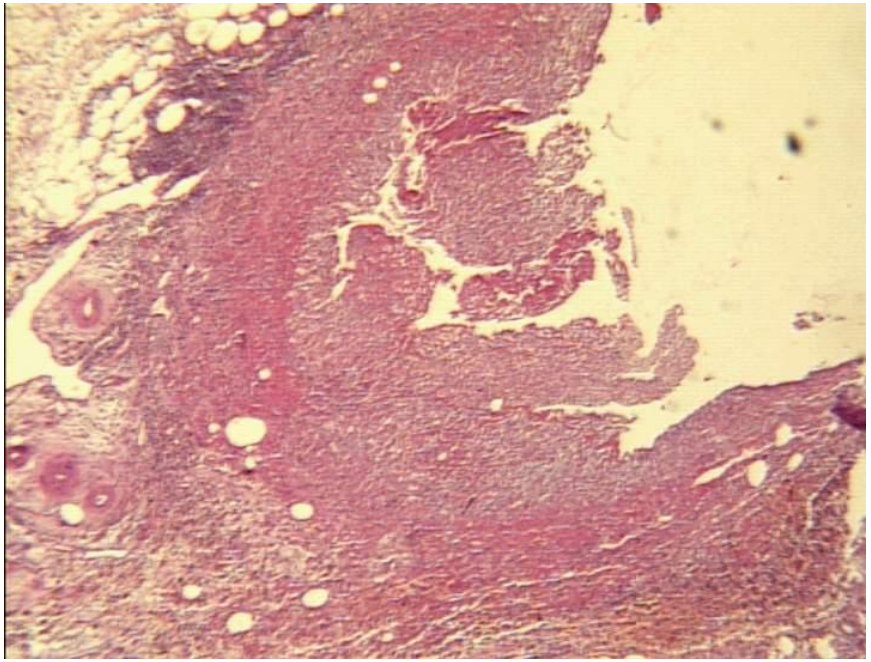




Diagnostic categories according to Banff-classification criteria

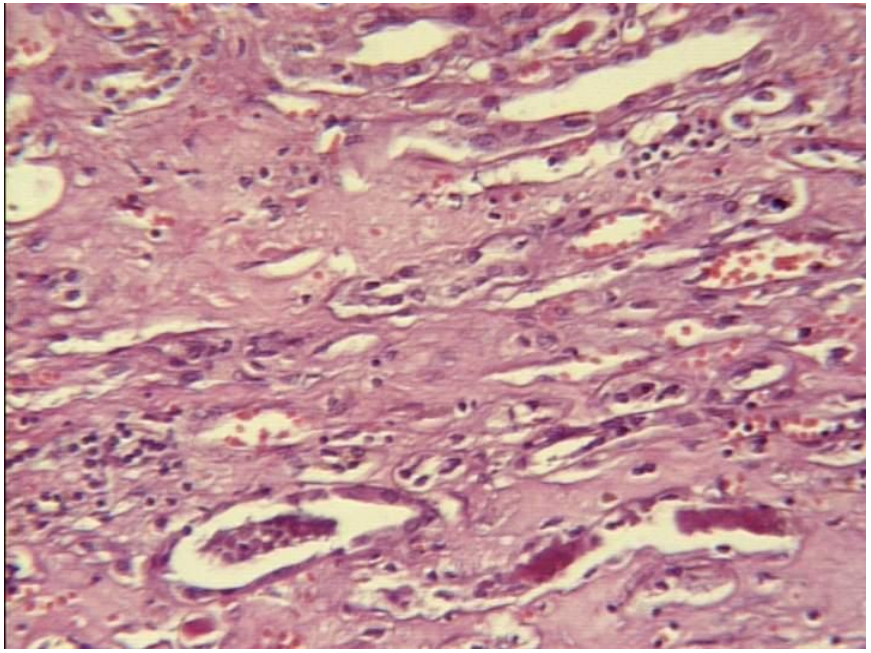
- **Severe acute rejection- grade 3: transmural arteritis**

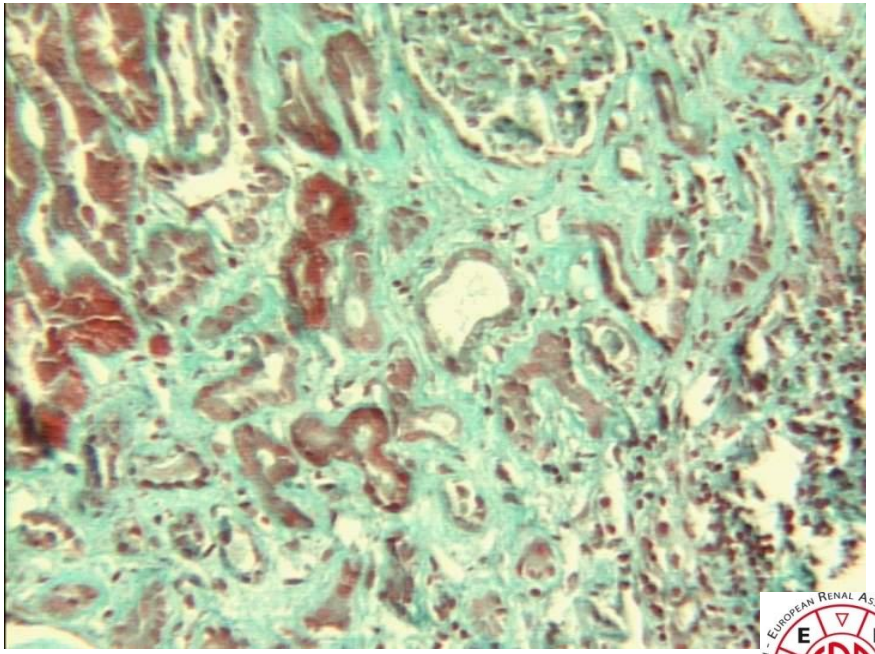




Diagnostic categories according to Banff-classification criteria

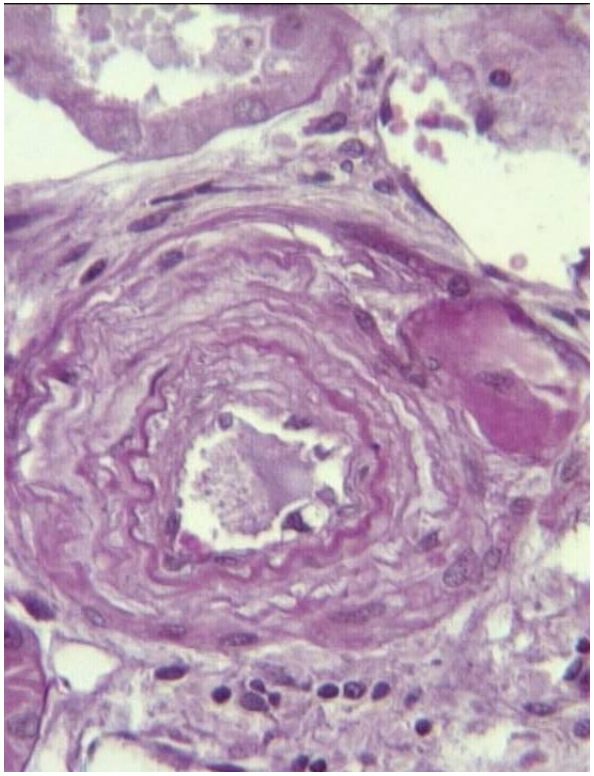
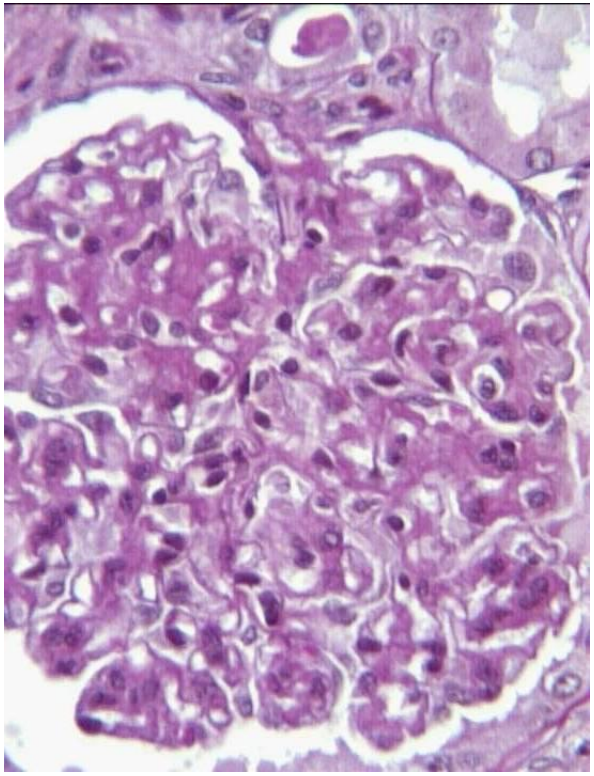
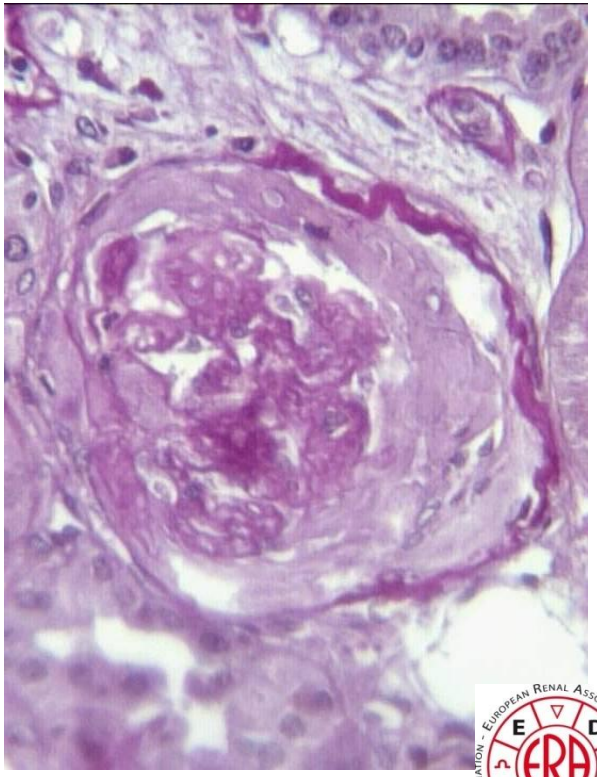
- **Chronic allograft nephropathy: tubular atrophy and interstitial fibrosis (mild, moderate, severe)**





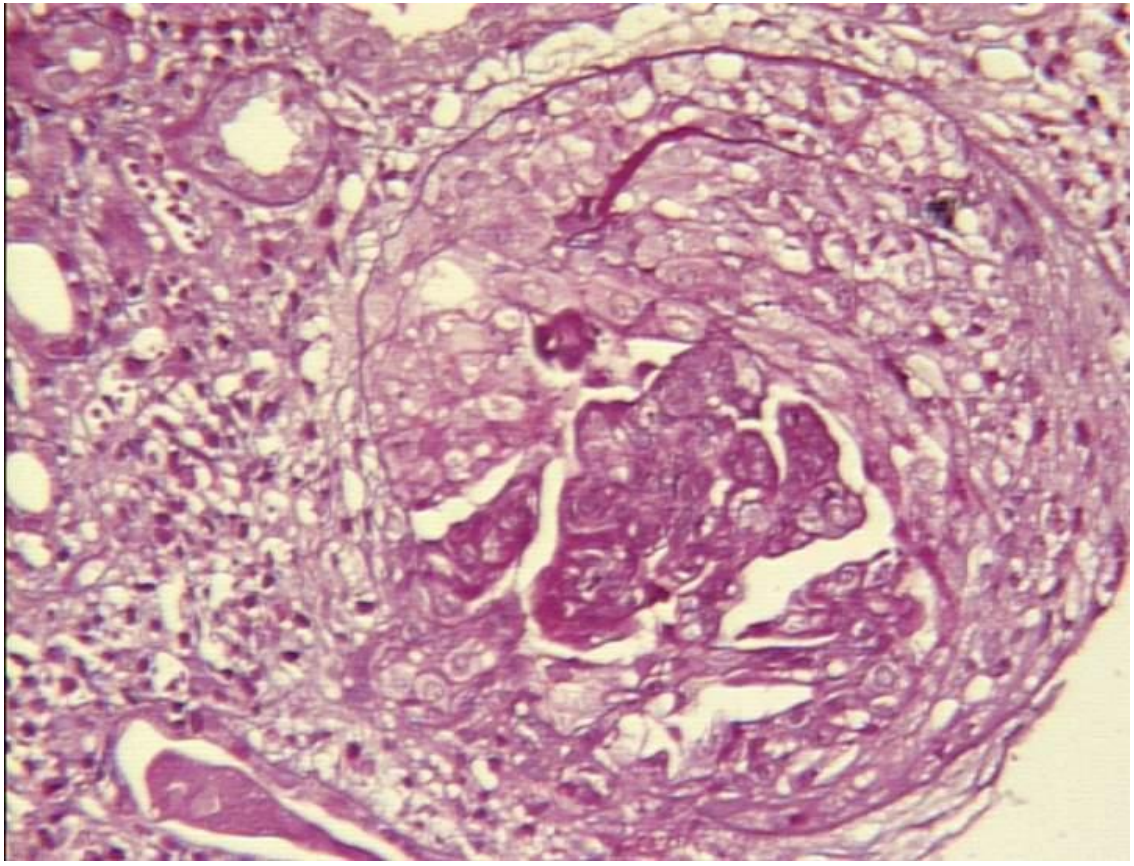
Diagnostic categories according to Banff-classification criteria

- **Chronic allograft nephropathy: intimal fibrosis, transplant glomerulopathy.**

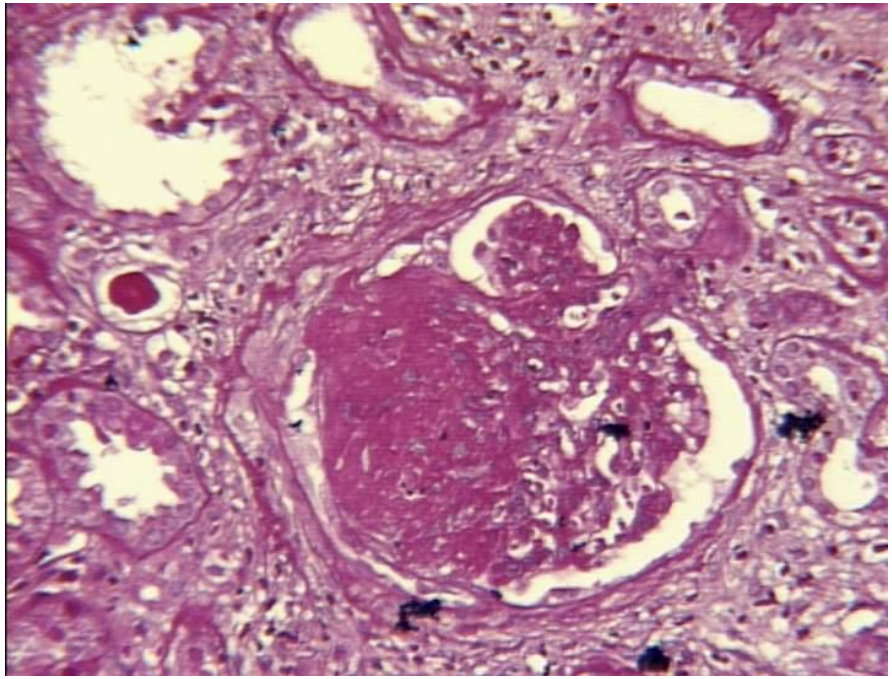
Diagnostic categories according to Banff-classification criteria

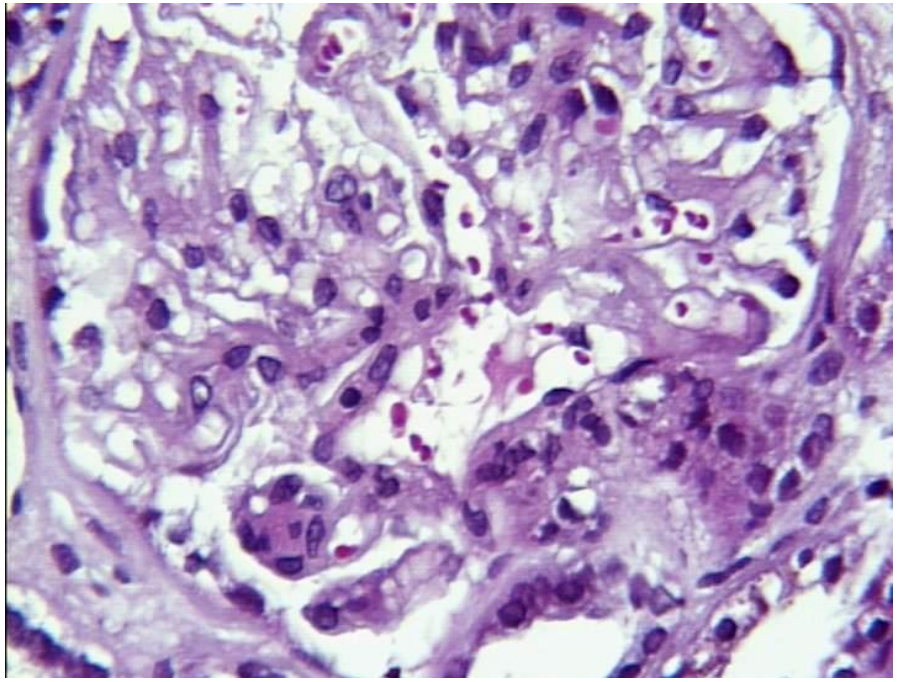
- **De novo glomerulonephritis**



Diagnostic categories according to Banff-classification criteria

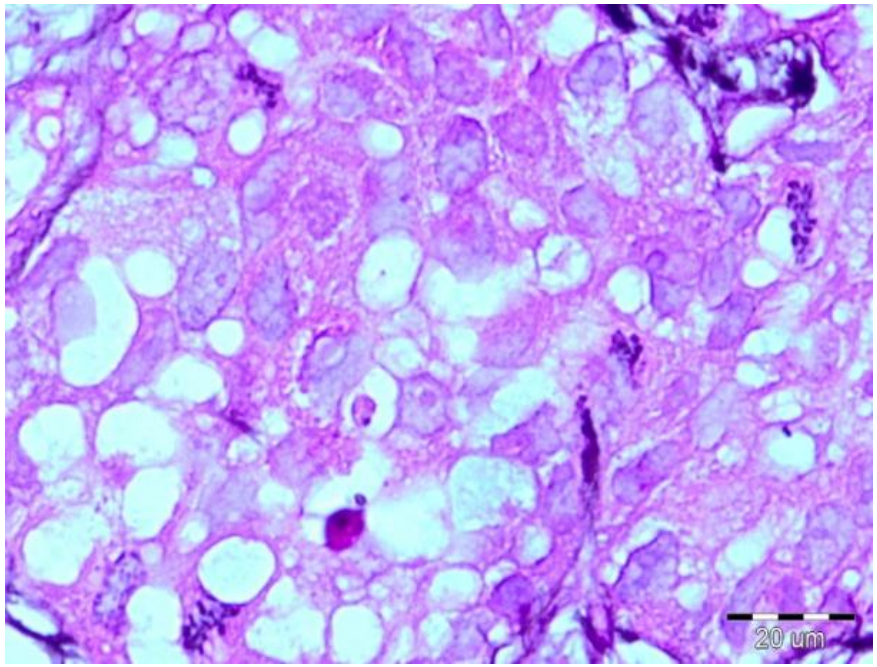
- **Recurrent disease**



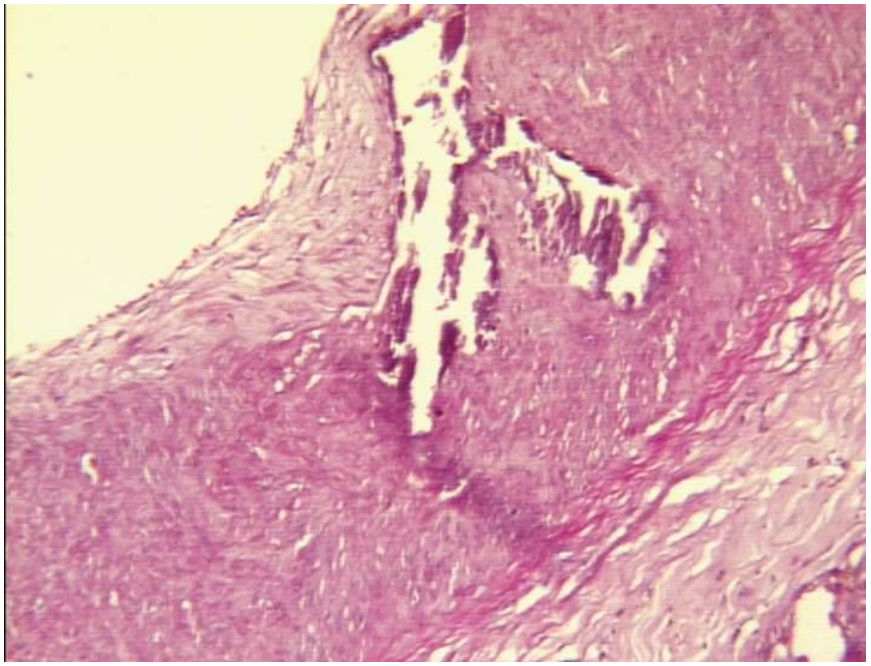


Diagnostic categories according to Banff-classification criteria

- **Transmission**



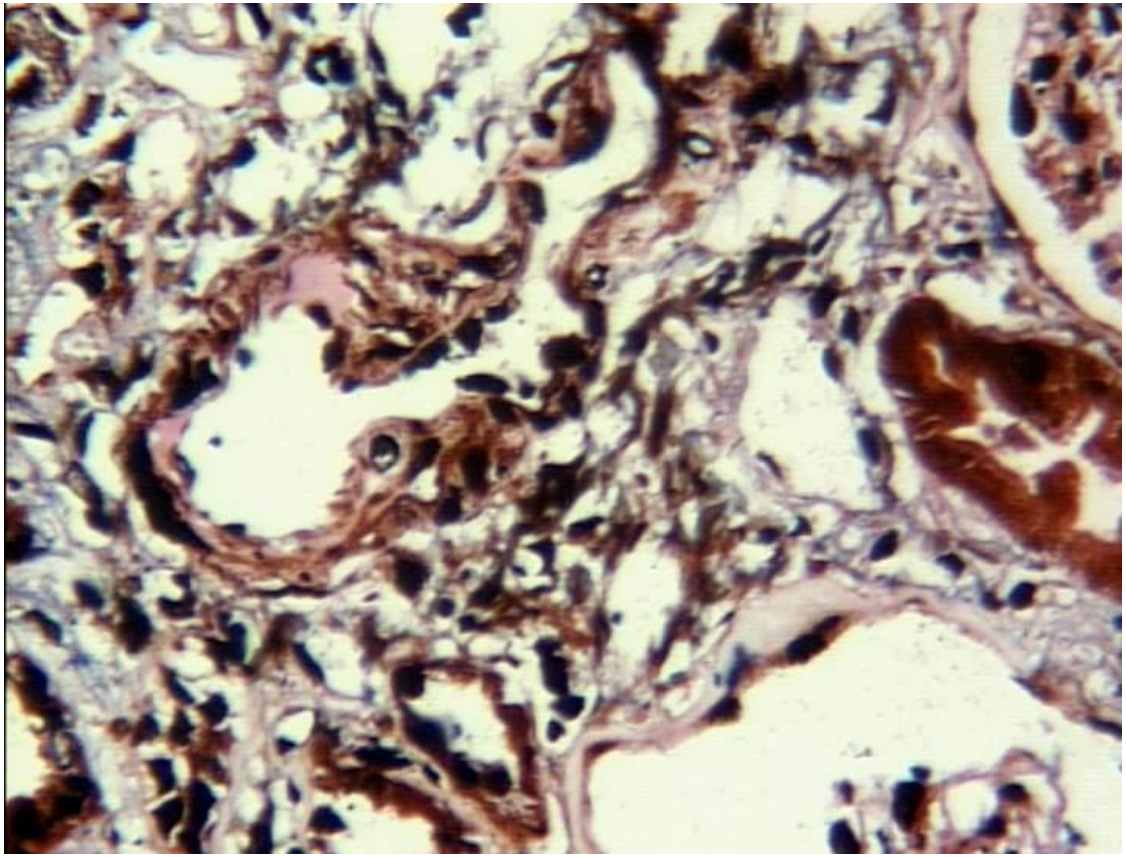
Transitional cell carcinoma from donor graft



Arteriosclerosis and calcinosis of the media

Diagnostic categories according to Banff-classification criteria

- **Cyclosporine toxicity**



Banff classification 07 – updates and future directions

American Journal of Transplantation 2008; 8: 753-760

1. **Normal**
2. **Antibody mediated changes (may coincide with categories 3, 4, 5 and 6) C4d deposition without morphologic evidence of active rejection.**
   - ***Acute antibody mediated reaction* (C4d+) –**
     acute active lesions (Type and grade);
   - ***Chronic active antibody mediated rejection* (C4d+)**
     chronic active lesions .
3. **Borderline changes suspicious for acute T-cell mediated rejection (may coincide with categories 2 and 5 and 6). There are foci of tubulitis (t1, t2 or t3) with mild interstitial infiltration (i0 or i1) or i2 and i3 with mild (t1) tubulitis.**

Banff classification 07 – updates and future directions

4. T cell mediated rejection (may coincide with cat. 2, 5 and 6)

- **Acute T cell mediated rejection (Type/Grade):**
  - **Grade 1A – significant interstitial infiltration (>25% of parenchyma affected, i2, i3) + moderate tubulitis(t2);**
  - **Grade 1B –significant interstitial infiltration (>25% parenchyma affected, i2, i3) and foci of severe tubulitis (t3);**
  - **Grade 2A Mild to moderate arteritis (v1)**
  - **Grade 2B – severe intimal aretritis (v2);**
  - **Grade 3 – transmural arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells (v3) + lymphocytic inflammation**

- **Chronic active T cell mediated rejection – chronic allograft arteriopathy (intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima).**

Banff classification 07 – updates and future directions

5. **Interstitial fibrosis and tubular atrophy - may include vascular and glomerular sclerosis.**

   - **Grade 1 – mild interstitial fibrosis and tubular atrophy without/with specific changes suggesting rejection.**
   - **Grade II - Moderate interstitial fibrosis and tubular atrophy**
   - **Grade III – Severe interstitial fibrosis and tubular atrophy with tubular loss**

Other –changes not considered to be due to rejection

Banff classification 07 – updates and future directions

American Journal of Transplantation 2008; 8: 753-760

1. Inclusion of: peritubular capillaritis grading (0,1,2,3);
2. C4d scoring (negative, mild, focal, diffuse);
3. Interpretation of C4d deposition without morphological evidence of active rejection;
4. Application of the Banff criteria to zero time and protocol biopsies;
5. Introduction of a new scoring for total interstitial inflammation (ti score);
6. Establishment of collaborative working groups addressing issues like isolated `v` lesion and introduction of omics technologies;
7. Future combination of graft biopsy and molecular parameters.

INTRODUCTION

- In Macedonia, most cases usually underwent biopsy at the time of graft dysfunction. A study of the role of the protocol biopsies at one and six months in patients with renal grafts has been done.

- In this study we analyzed the main histopathological changes in the protocol biopsies from grafts with stable renal function, taken at one and six months using Banff Classification 1997, for a three year period.

- We also determined proliferative and apoptotic indexes in renal protocol biopsies to obtain insights into their role in CAN changes.

MATERIALS AND METHODS

- A total of 28 paired biopsy specimens from allografted kidneys performed at 1 and 6 months, were collected at the Medical faculty, University of Skopje, between October 2002 and April 2004.
- All specimens were fixed in 10% formalin, embedded in paraffin
- Paraffin sections were stained with H&E, PAS, Masson trichrome and Silver methenamine-PAS. Biopsies were considered adequate when they contained more than 7 glomeruli and at least one artery.
- Additional immunohistochemical staining for Ki67 by LSAB immunoperoxidase was done, as well as ApoPtag in situ hybridization for the detection of apoptosis.

MATERIALS AND METHODS

- The immunosuppressive regimen consisted of methylprednisolone and Daclizumab as induction therapy, and cyclosporine, prednisolone and mycophenolate mofetil as maintenance post-transplant immunosuppression.

- Clinical parameters analyzed included age, sex, source of donor, serum urea/creatinine level, urine volume and presence of proteinuria. Histological diagnosis was made according to the criteria of the Banff working classification.

- In present study we included cases with serum creatinine <200 μmol/L and proteinuria <1g/24 hours at the time of the first biopsy, which was defined as “stable” graft function.

RESULTS

- The mean age of the recipients was 35.2+/-8.3 years
- Male to female ratio was 3/1.
- The mean living donor age was 58.5+/-13.4 years.
- Histopathological diagnosis included six specimens (21,4%) with normal findings in the 1 and 6 months biopsies.
- Borderline changes were found in 10/28 (35,7%) and 10/28 (35,7%) at 1 and 6 months biopsies, respectively.

RESULTS

- **Signs of acute rejection were found in 13/28 (46,4%) and 12/28 (42,8%) cases, at 1 and 6 months biopsy respectively: mild AR (5/28), moderate (6/28) and severe (2/28) in the first month, and mild 4/28 and moderate AR 12/28 in the sixth months biopsy.**

- **There was significant increase of CAN in the second allograft biopsy after six months:**
  - In the first biopsy we found mild degree of CAN in 14 specimens (50%) and moderate in 2 specimens (7,1%).
  - On second biopsy CAN was detected in 23 cases (82%) (11 with mild CAN and 12 with moderate CAN).

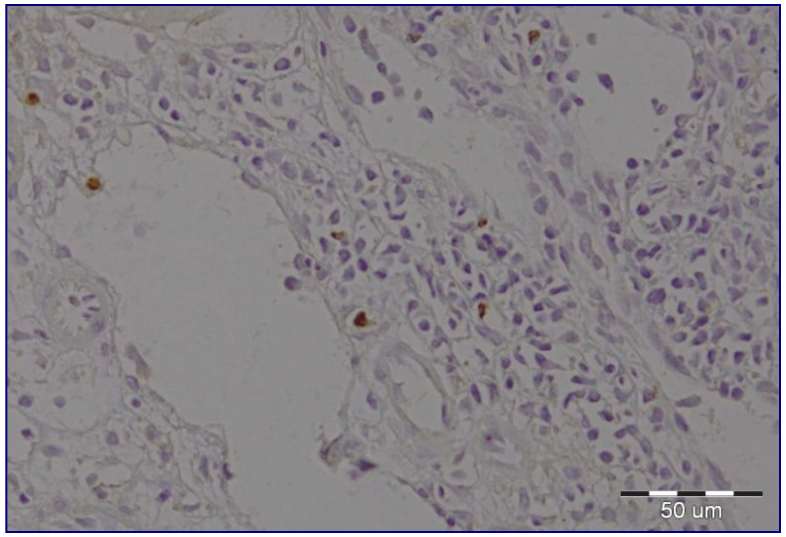
RESULTS

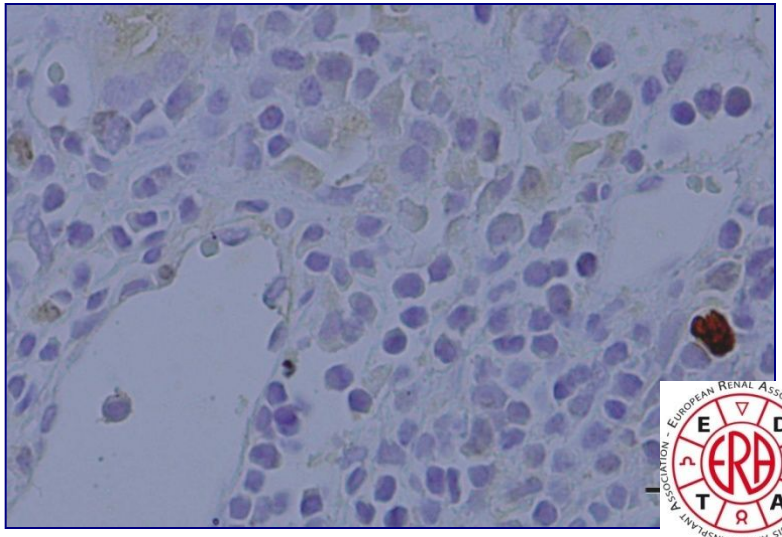
- It is of interest that in three cases there were signs of cyclosporine nephrotoxicity, although the blood concentrations were within normal ranges. Cyclosporine nephropathy showed typical arteriolopathy with nodular hyaline subintimal deposits.

- We correlated these findings with the serum creatinine levels (sCr): We found significantly increased levels of sCr at 6 months after transplantation, while the calculated creatinine clearance (cCrcl) and proteinuria were significantly lower compared to the one month values for the respective group.

RESULTS

- **Immunohistochemical study for cell proliferation by Ki 67 showed greater proliferative index in the second biopsies taken at 6 months (3-4+ve cells per 10 HPF).**
- **Proliferation was almost absent in the biopsies taken at 1 month after transplantation.**

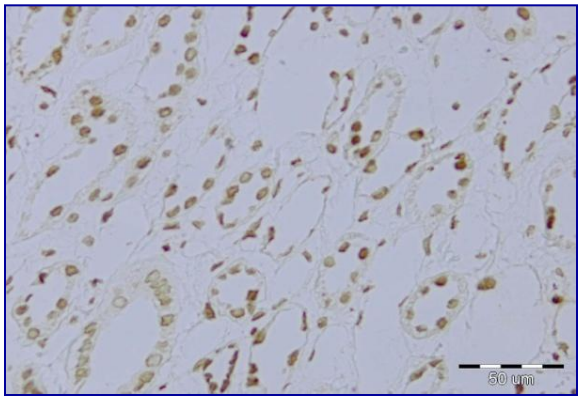


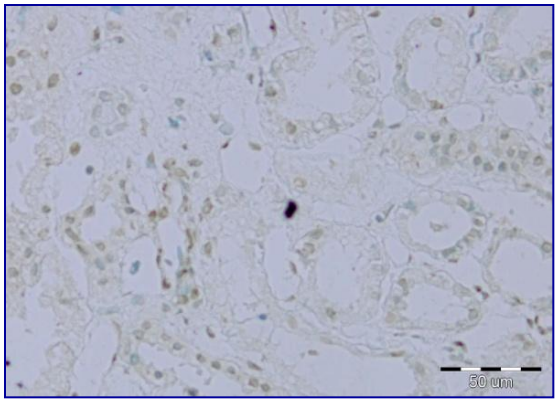


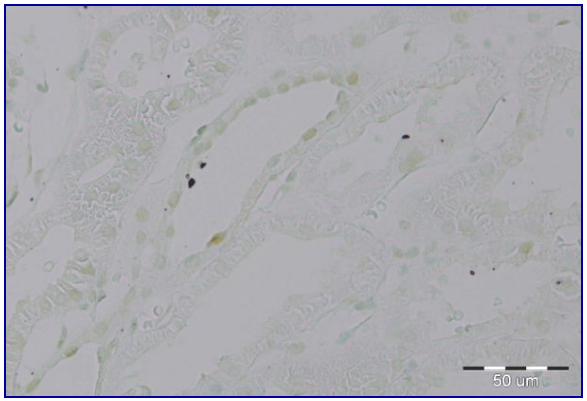
RESULTS

- **Evaluation of the apoptosis showed significant number of cells that expressed apoptosis markers (>30 cells per 10 HPF) in the biopsies taken at 1 month after transplantation.**

- **At the 6 months biopsies the level of apoptosis has significantly decreased (<5 cells per 10 HPF)**







DISCUSSION

- **We demonstrated histopathological findings in grafted kidneys, which clinically showed adquate renal function at the time of biopsy. To select specimens, we arbitrarily set up the criteria of serum creatinine less than 200 μmol/L and proteinuria less than 1g/24 hours at the time of the first biopsy, which was defined as "stable" graft function.**

- **Our results showed presence of high percentages of BR and AR in allograft biopsies, which means they do not necessarily cause clinically recognizable graft dysfunction. Higher percentages of BR and SR in this study, compared to previous reports, might be due to the different sampling time for the biopsies, as well as to the lower number of patients included in the study.**

DISCUSSION

- **Study of protocol biopsies from stable grafts had revealed an unexpectedly strong correlation between the subsequent decline in renal function and the presence of acute histologic features, such as tubulitis and lymphocytic infiltration; this gives some support to the concept of "subclinical acute rejection" in the pathogenesis of chronic graft damage or chronic allograft nephropathy (CAN).**

- **In favor of this concept is the significant increase of CAN in the second biopsy taken at 6 months after transplantation, in our study.**

DISCUSSION

- **Findings of recurrent disease and cyclosporine nephrotoxicity are important because of the further treatment strategy of such patients.**

- **High level of apoptosis expression in early protocol biopsies shows that apoptosis might be one of the pathways in the development of CAN, together with epithelial-mesenchymal transformation.**

CONCLUSION AND RECOMMENDATIONS

There are three possible stategies:

1. **No biopsy: This is the default position of many transplant units world wide and assumes that either SCR is unimportant and can be ignored, or that it is relevant but can be controlled by high-dose anti-rejection therapy. This may result in increased rates of BK nephropathy, nosocomial infections and post-transplant lymphoproliferative disease.**

CONCLUSION AND RECOMMENDATIONS

There are three possible strategies:

2. Biopsies in high-risk individuals: Although it is cheaper than universal biopsy programs, practical disadvantages include patient selection (who is `high risk`?) and implementation (individual recipients may feel unfairly selected or unselected). While individual selection is easy at the extremes of immunological risk, the difficulty arises with the large number of intermediate risk individuals-where accurate prediction is imperfect.

CONCLUSION AND RECOMMENDATIONS

There are three possible strategies:

3. Universal biopsy policy:

Protocol biopsies are valuable to determine presence of changes indicating acute or chronic rejection which impact on the evolution of renal allograft. They are also important for revealing other lesions that might compromise renal allograft function.

- Advantages include simplicity of implementation; detection of unsuspected SCR in low risk individuals; early detection of other diagnoses (BK nephropathy, transmission).

- Disadvantages include costs, consumption of clinical and pathology resources, total number of adverse events incurred – which would increase with higher throughput programs, despite a low per procedure risk.

CONCLUSION AND RECOMMENDATIONS

- **SCR results in chronic tubulointerstitial damage, impaired renal dysfunction and reduced graft survival. It is relatively common and easily and safely diagnosed by protocol biopsies. Corticosteroid treatment in a single randomized clinical trial and other cohort studies demonstrated improved structural, functional and graft survival outcomes.**

- **Hence, one could make a clinical screening strategy – either as protocol biopsy in all recipients, or in high-risk individudals only, as an alternative to blanket use of heavy immunosuppression.**

- **This decision is both a clinical and an economic decision, influenced by the prevalence of SCR and potential gains of treatment, against costs and resource utilization.**

Nankivell BJ et al: American Journal of transplantation 2006; 6: 2006-2012

THANK YOU FOR YOUR ATTENTION