HLA and Non-HLA Antibodies in Transplantation and their Management

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OSPEDALE PEDIATRICO

Hystory I

- 1960 “donor specific antibodies” (DSA): first suggestion for a possible role in deteriorating renal function
- 1970 (Jeannet) – worse graft outcome when DSA are present
- 1990 (Halloran) - humoral rejection is clearly identified. Clinics and pathology are defined

Hystory II

- 1991, 1993 Feucht identifies “C4d” (byproduct after C4 metabolism) in peritubular capillaries of “high immunonologic risk” patients
- It is then proposed as a specific marker for humoral rejection

Hystory III

- 1999 Collins: C4d staining within peritubular capillaries is associated to circulating antibodies against class I and II HLA donor antigens

C4d vs donor specific antibodies

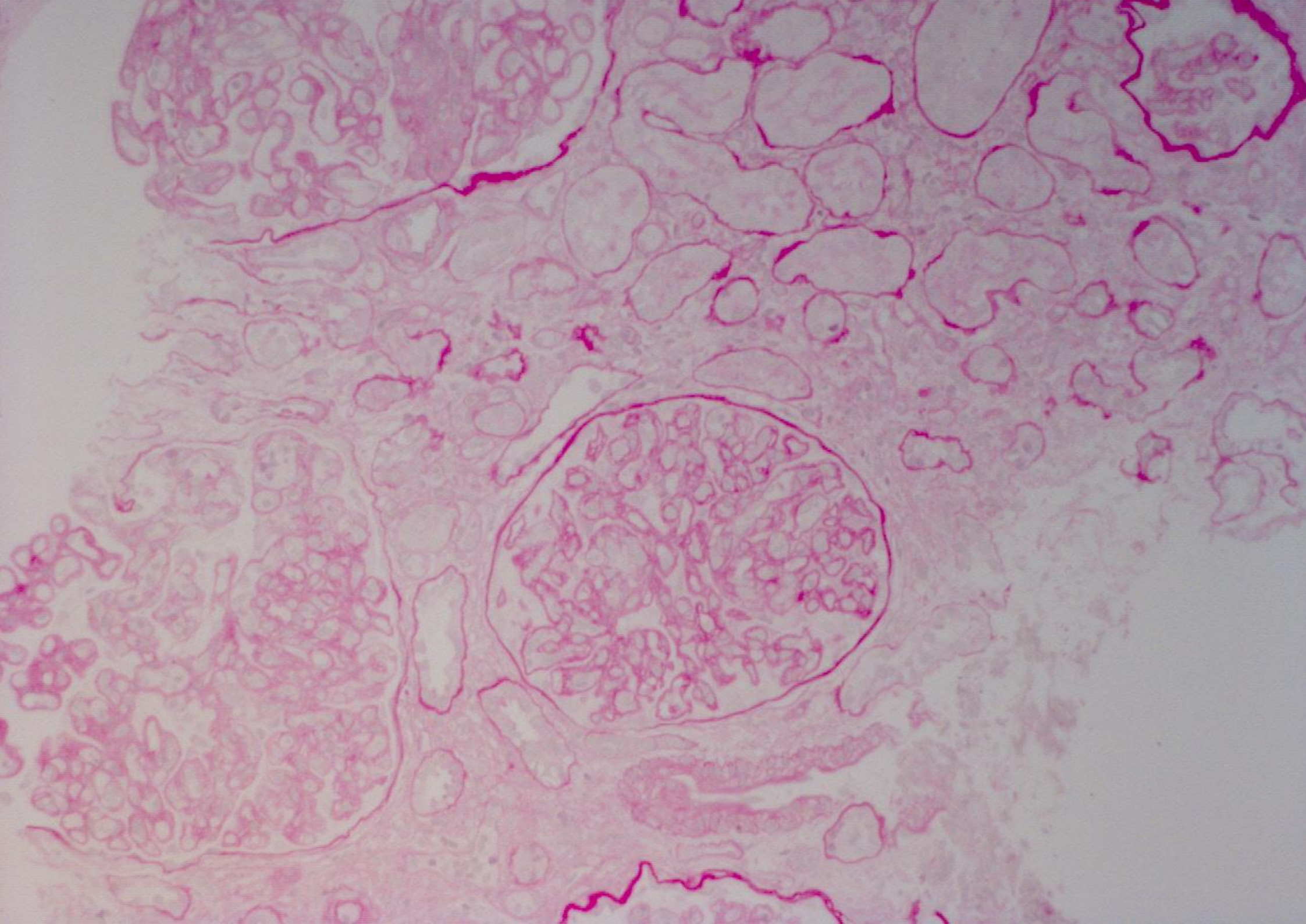
	N	DSA
C4d+	20	18 (90%)
C4d-	47	1 (2%)

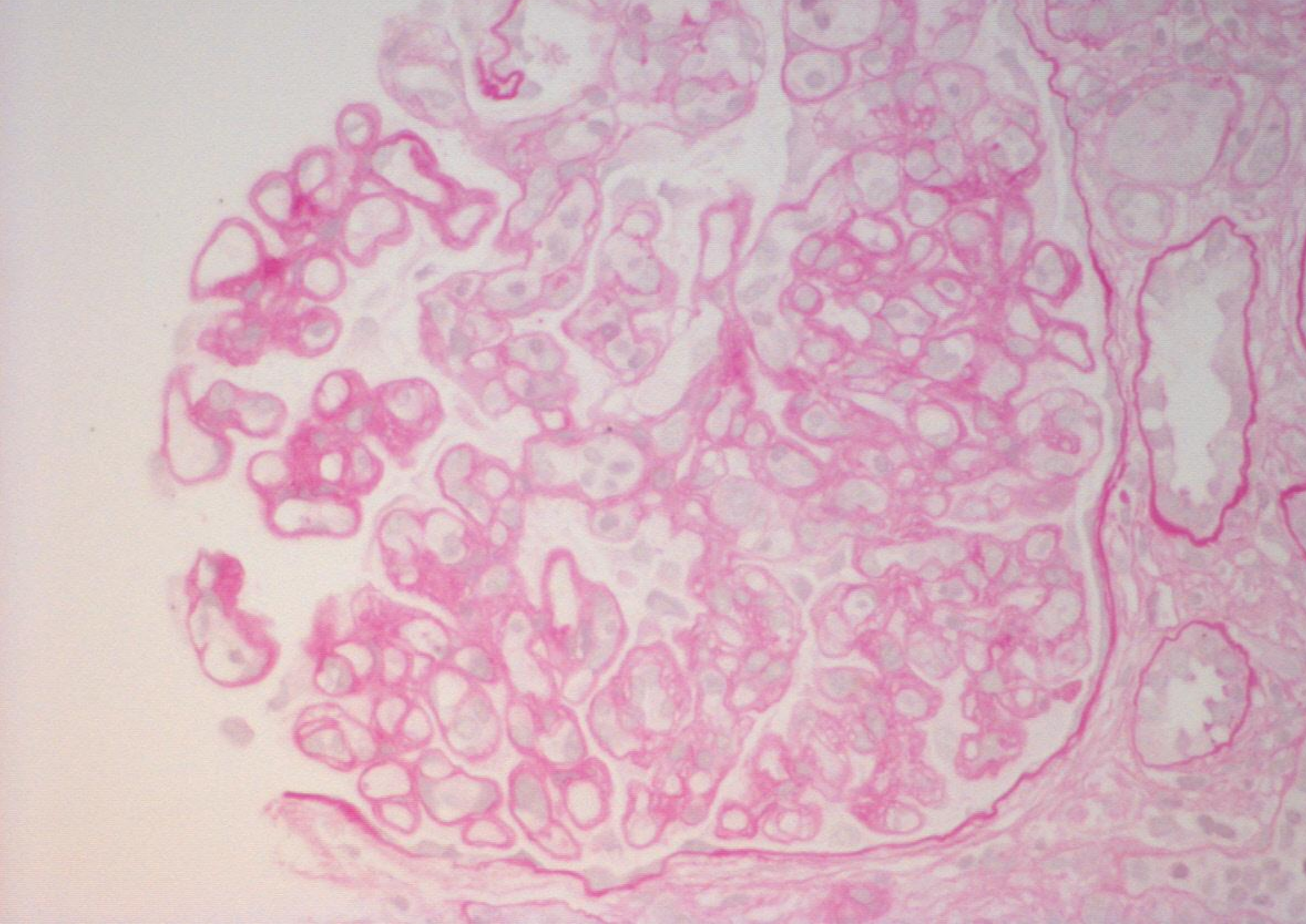
Mauyyedi JASN 2002

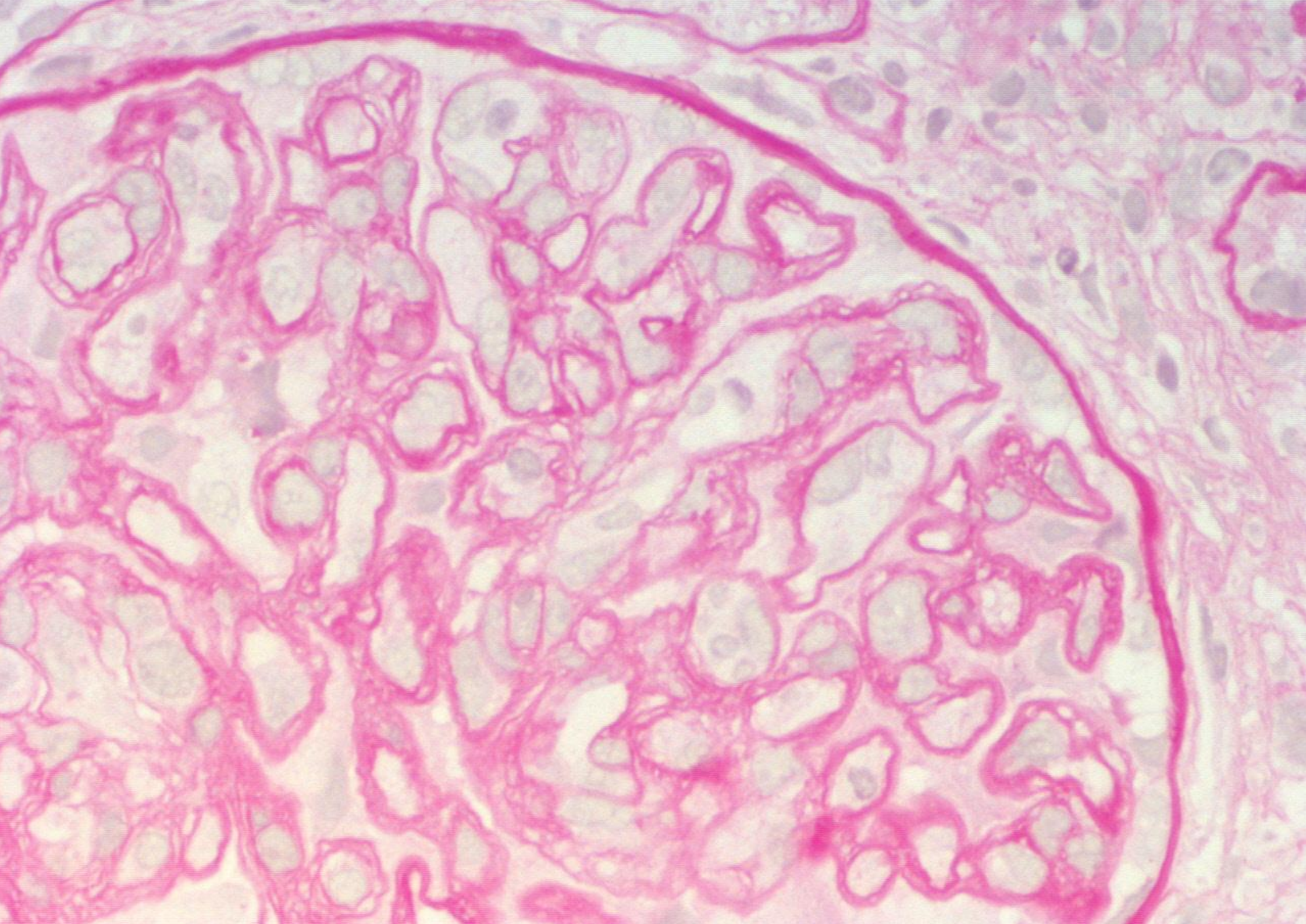
Antibody mediated rejection

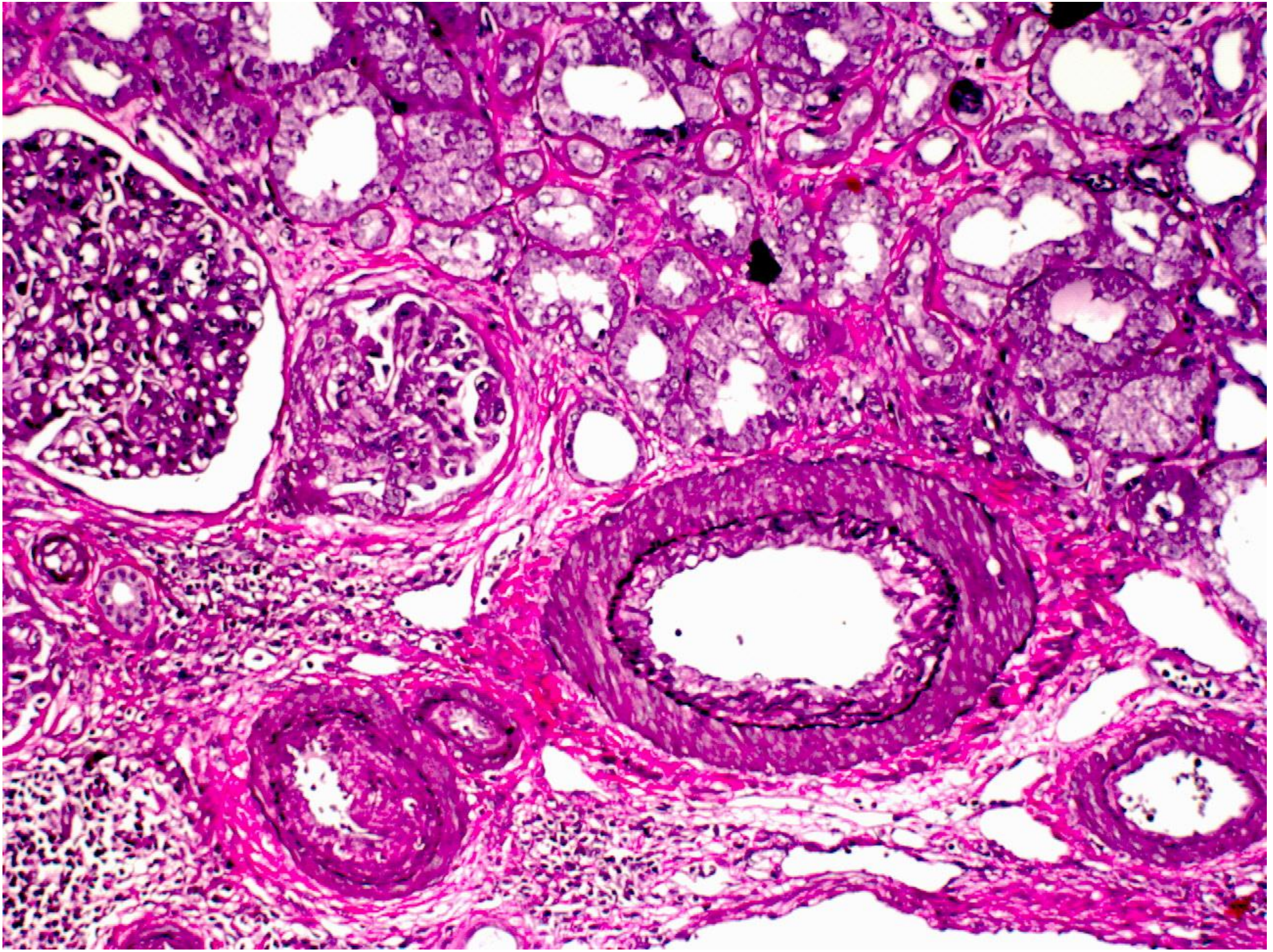
- Histology
  - acute tubular injury,
  - neutrophils and/or mononuclear cells in peritubular capilaries and/or glomeruli and/or capillary thrombosis, fibrinoid necrosis/intramural or transmural inflammation in arteries
- immunopathologic evidence: C4d or immunoglobulins deposition in peritubular capilaries
- serologic evidence: anti-donor antibodies

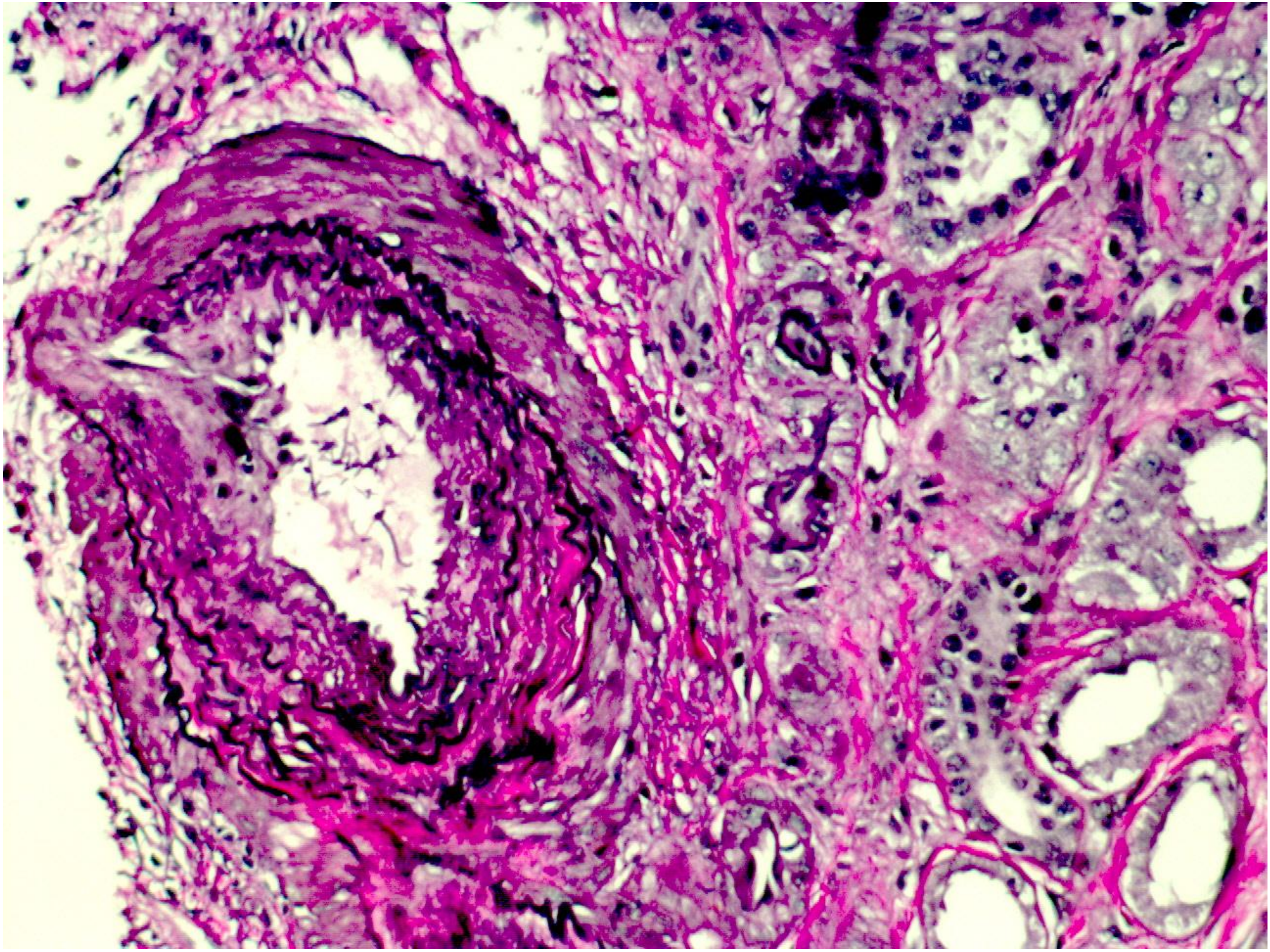
Racusen AJT 2003

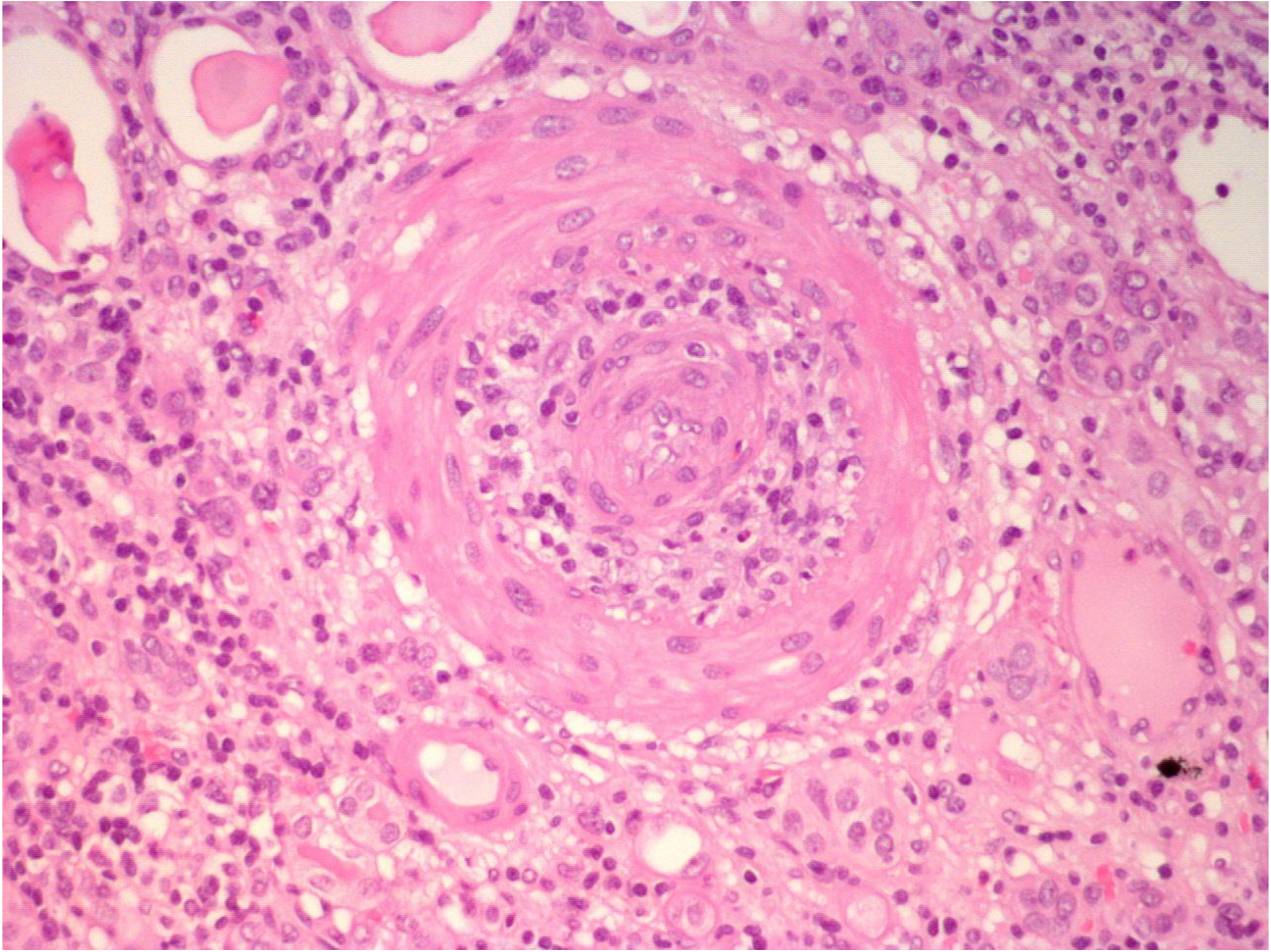




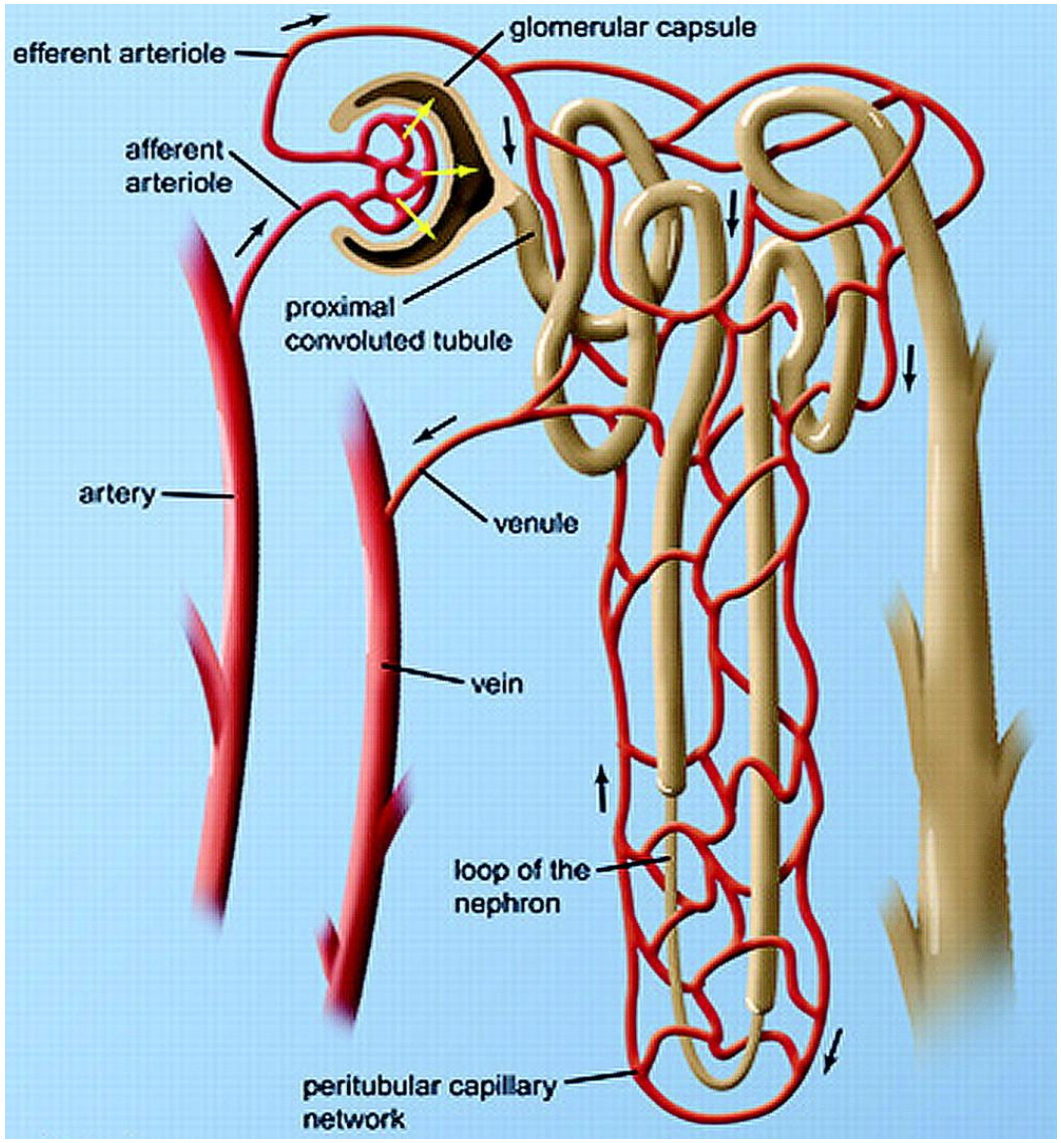




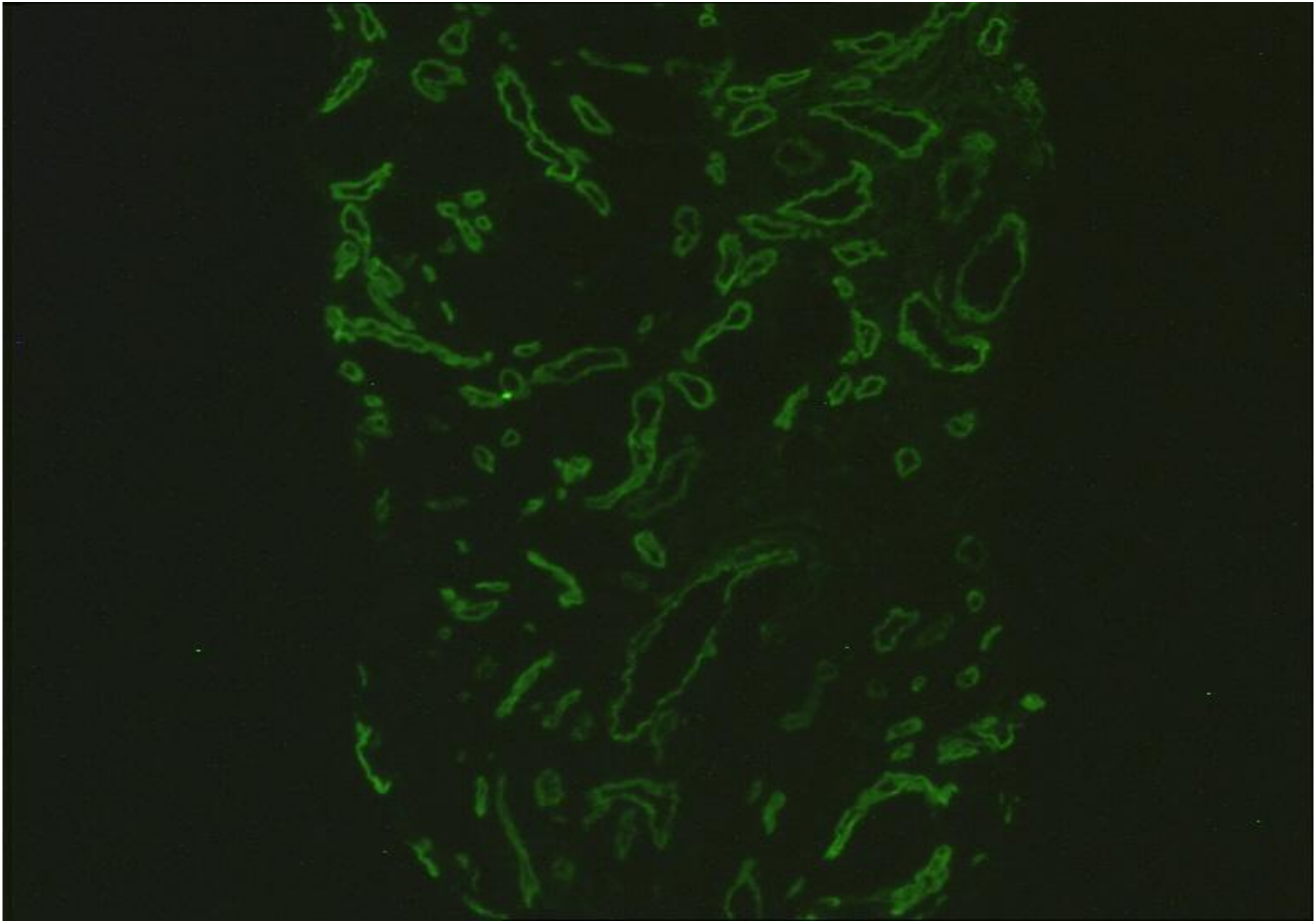


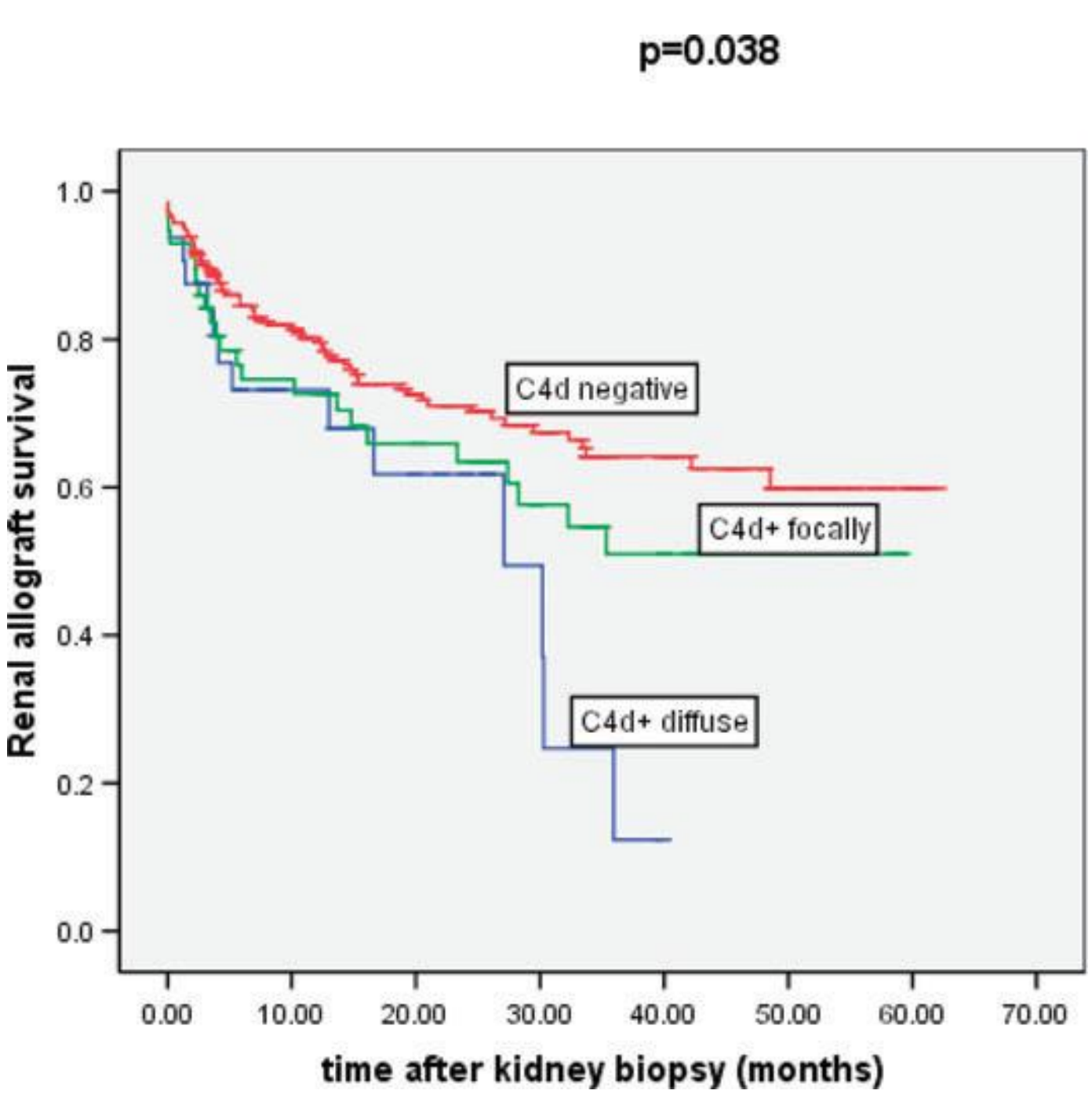


The microvasculature of the nephron.



Nangaku M JASN 2006;17:17-25





American Journal of Transplantation 2009; 9: 812–819

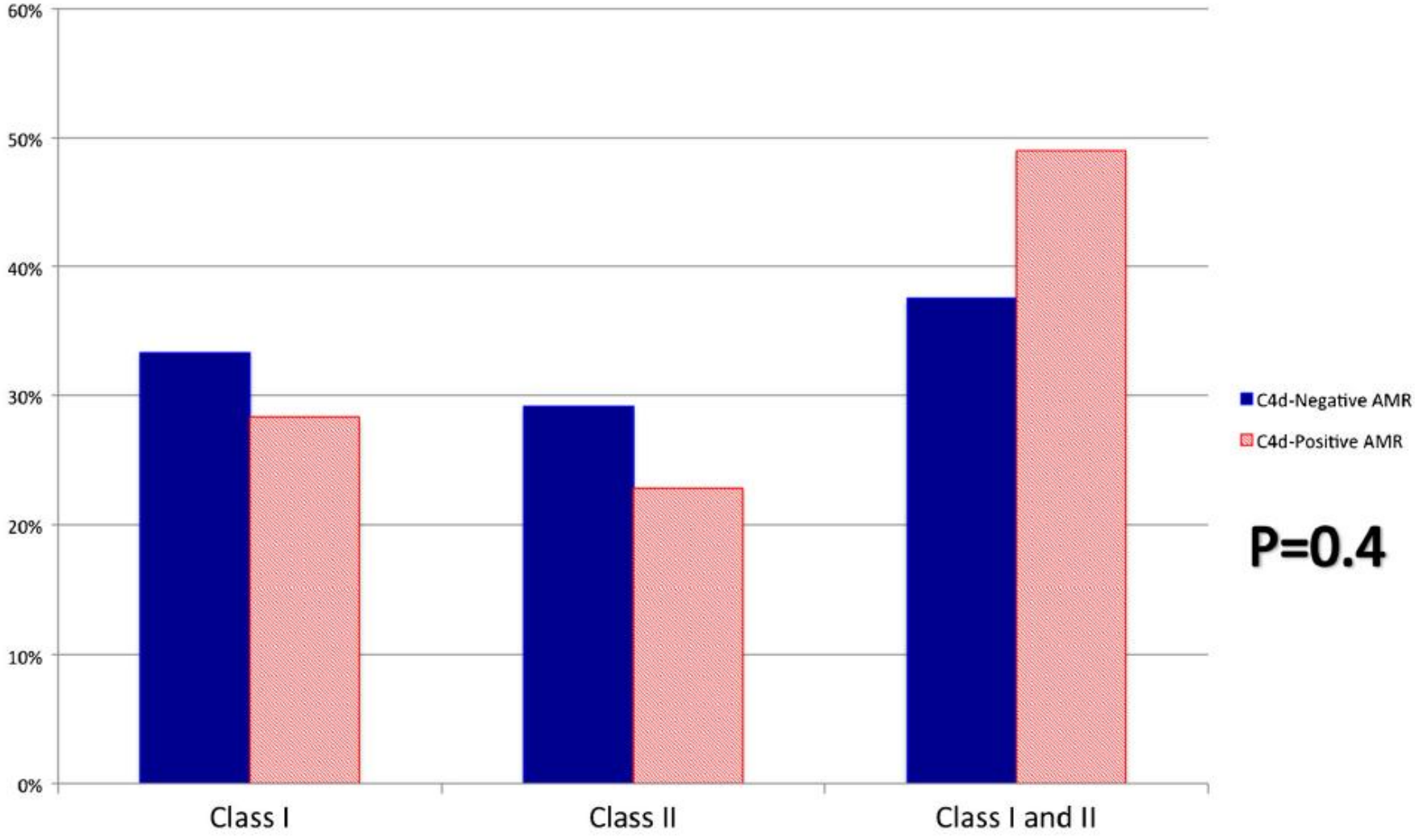
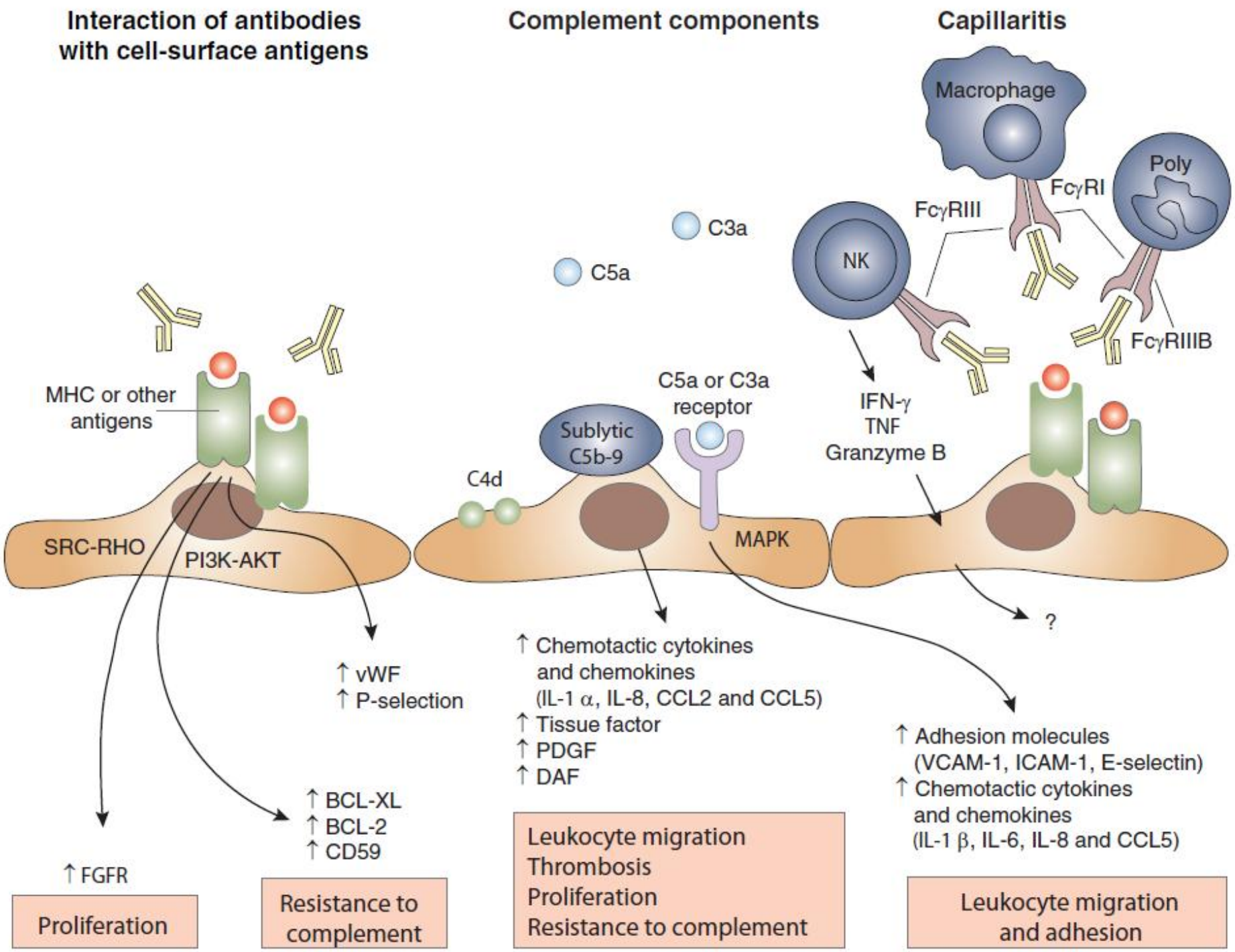


Figure 1: HLA DSA by AMR type at the time of AMR-defining biopsy. AMR, antibody-mediated rejection.

Am J Transpl 2016; 16: 213–220

	Hazard of graft loss (95% CI) compared with AMR-free matched controls	p value
C4d-negative AMR	2.56 (1.08–6.05)	0.033
C4d-positive AMR	3.70 (2.47–5.54)	<0.001

Am J Transpl 2016; 16: 213–220



Am J Transpl 2014; 14: 255–271

DONOR SPECIFIC ANTIBODIES

question

- are all donor antibodies directed against HLA antigens?

Antibody mediated rejection

- Preformed / *de novo* antibodies

  Against class I or II anti HLA antigens

  Ab vs Non-HLA antigens:

  – MICA: Major-histocompatibility-complex class I–related chain A antigens

  – AT_1R-AA : Agonistic antibodies against the Angiotensin II type 1 receptor

  – Others (Anti-endotheline type 1 receptor, antiperlecan antibodies,….)

What are MICA?

- MICA = Major-histocompatibility-complex class I–related chain A (MICA) antigens
- are surface glycoproteins with functions related to innate immunity .
- are expressed on endothelial cells, dendritic cells, fibroblasts, epithelial cells, but not on peripheral-blood lymphocytes.
- Therefore, antibodies directed against MICA are not detected with the methods generally used for cross-match.

N Engl J Med 2007;357:1293-300.

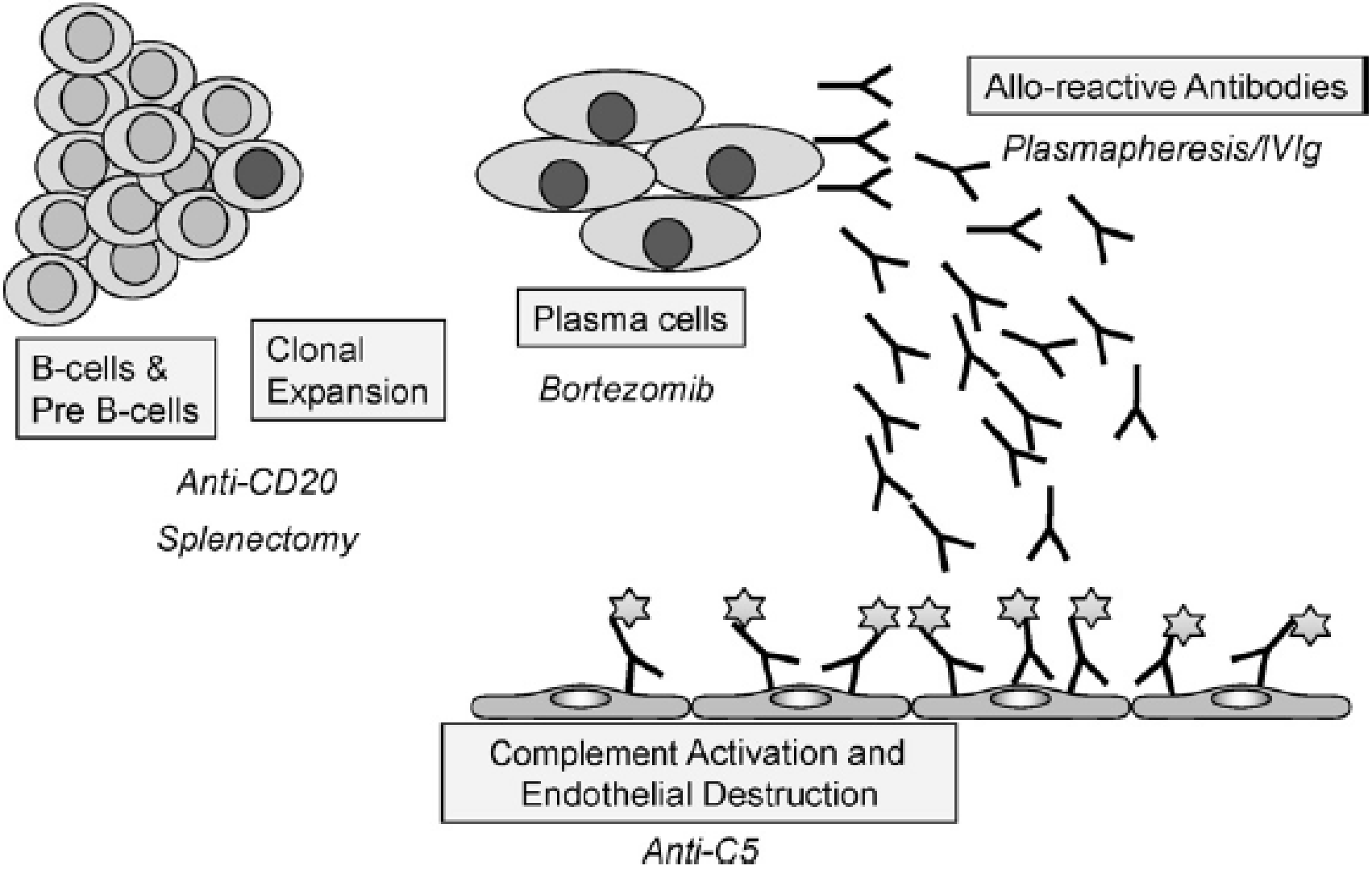
Agonistic antibodies against the Angiotensin II type 1 receptor (AT1R-AA)

- Classically reported a rejection with severe hypertension
- Hystology: endarteritis, transmural arteritis and/or fibrinoid vascular necrosis (Banff IIb or Banff III rejection)
- Is it a ‘‘true-rejection’’ or an autoimmune phenomenon triggered in the permissive allogeneic and post-ischemic inflammatory enviroment?

Dragun N Engl J Med 2005; 352: 558–69

TREATMENT

Therapeutic Approaches For Crossing Antibody Barriers to Solid Organ Transplantation



R.A. Montgomery et al. / Seminars in Immunology *23 (2011) 224– 234*

Immunoglobulin

- 20 highly sensitized patients (PRA 77±19%) were enrolled and received treatment with intravenous immune globulin and rituximab
- 16/20 received a transplant.
- At 12 months, the mean serum creatinine level was 1.5±1.1 mg/dl (133±97 μmol/l)
- mean survival rates of patients and grafts were 100% and 94%, respectively

N Engl J Med 2008; 359: 242–251.

Immunoglobulin

- double-blind placebo controlled trial of high-dose IVIg-based desensitization
- compared high dose IVIg alone vs. high-dose IVIg plus rituximab in patients with PRA > 80% (clinicaltrials.gov
- study #NCT01178216; 42).
- IVIg (2 g/kg weeks 1 and 4) and rituximab
- (1 g given at week 2).
- The trial was originally designed to enroll 90 patients, but was halted by the DSMB after only 15 patients were enrolled because of high AMR and allograft loss rates.

Am J Transplant 2013: 13(Suppl 5): 76 abstract #153

Immunoglobulin

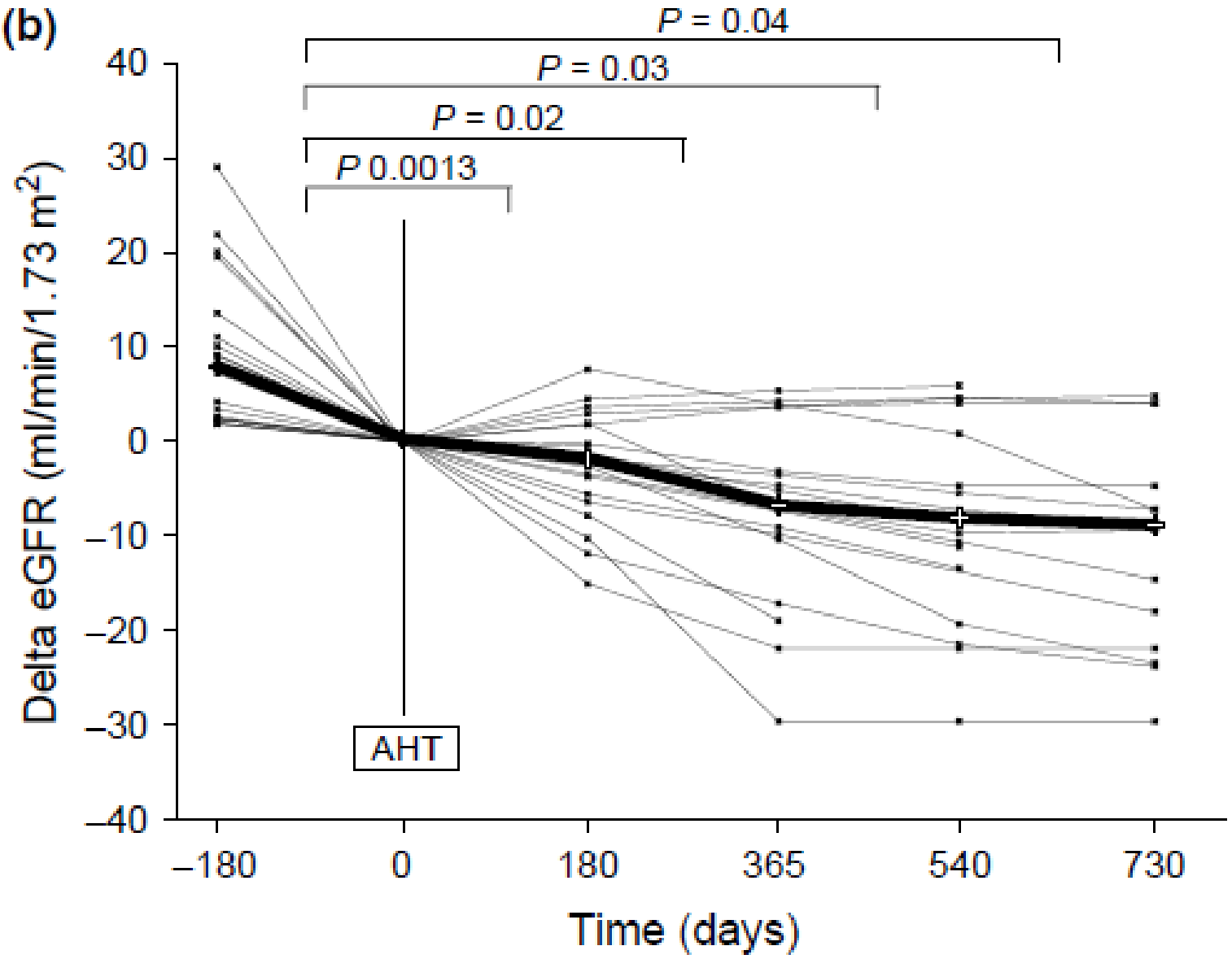
- Two additional study have not been able to reproduce the potential of immunoglobulin in reducing anti-HLA antibody levels and improving transplantation rates , specifically in patients with PRA >80%

Transplantation 2012; 94: 345–351
Transplantation 2012; 94: 165–171.
Am J Transpl 2014; 14: 255–271

rituximab

Rituximab is a chimeric antibody recognizing the cell surface marker CD20, which is expressed at most stages of B-cell development except the very early stages, but not on plasma cells

IVIG and rituximab: pediatric patients



Billing et al: Transpl Int. 2012 ;25:1165-73.

Rituximab and desensitization: review

Study (yr) country	No. of patients (RTX/non-RTX)	Study period, mo	Treatment regimen (RTX/non-RTX)	Baseline IS	T-cell induction therapy	Patient survival
Retrospective cohort studies						
Hyodo (2011)* Japan (*34*)	122 (29/31/62)	60	RTX+MMF/SPX+MMF/SPX+AZA	Not fully reported	Not reported	Not reported
Aikawa (2011)* Japan (*35*)	111 (16/95)	36	RTX+PE or PP/SPX+PE or PP	TAC or CsA, MMF or AZA+CS	BXM[c]	No difference
Tanabe (2007) Japan (*17–21, 36–41*)	102 (57/45)	24	RTX+PP/SPX+PP	TAC, MMF+CS	BXM	No difference[d]
Ashimine (2014) Japan (*22*)	81 (30/51)	36	RTX+PP/SPX+PP	TAC or CsA+MMF or MZR	BXM	No statistical comparison
Harada (2013)* Japan (*42*)	70 (46/24)	60	RTX+PP/SPX+PP	TAC, MMF, or AZA+CS	BXM or ALG	No statistical comparison
Charif (2013)* UK (*43*)	63 (24/39)	36	RTX+PE/ALZ+PE	TAC+CS±MMF[g]	DAC (RTX group only)	No difference
Nakagawa (2011)* Japan (*44*)	61 (42/19)	36	RTX/SPX	TAC or CsA, MMF+CS[h]	BXM (RTX group only)	No difference
Montgomery (2009) USA (*23*)	60 (3/15/14/28)	60	RTX, IVIg, PP+SPX/RTX, IVIg+PP/SPX, IVIg+PP/	TAC, MMF+CS	DAC	Not reported
Gloor (2005) USA (*24*)	34 (11/23)	24	RTX, IVIg+PP/SPX, IVIg+PP	TAC, MMF+CS	ATG	No difference
Waigankar (2013) India (*25*)	26 (7/19)	12–18	RTX, PP+IVIg/SPX, PP+IVIg	TAC, MMF+CS	Not reported	No statistical comparison

Transplantation 2014;98: 794-805

Rituximab: conclusions

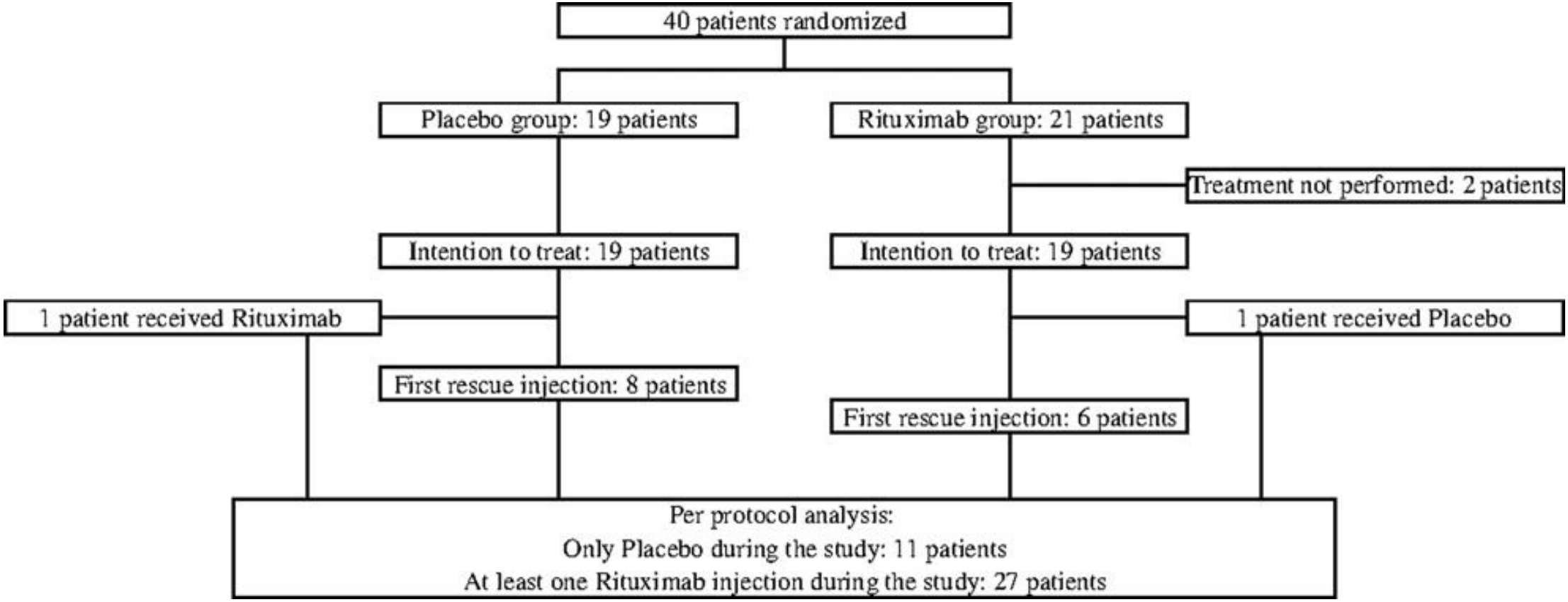
- no strong evidence exists to support superior patient and graft outcomes with rituximab
- optimal dose and number of infusions of rituximab is still unknown
- the diversity of therapeutic protocols, using a variety of complex medications, means that it is difficult to confidently attribute outcomes solely to the administration of rituximab

Transplantation 2014;98: 794-805

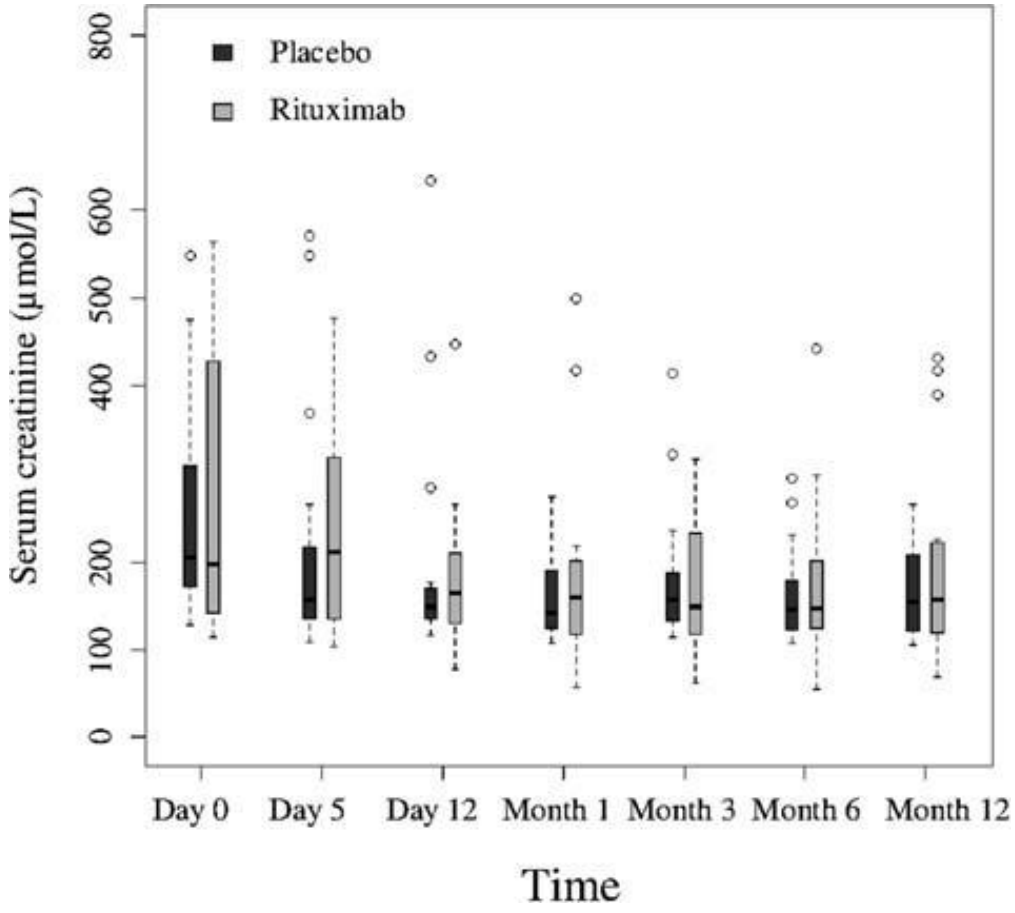
One-year Results of the Effects of Rituximab on AMR

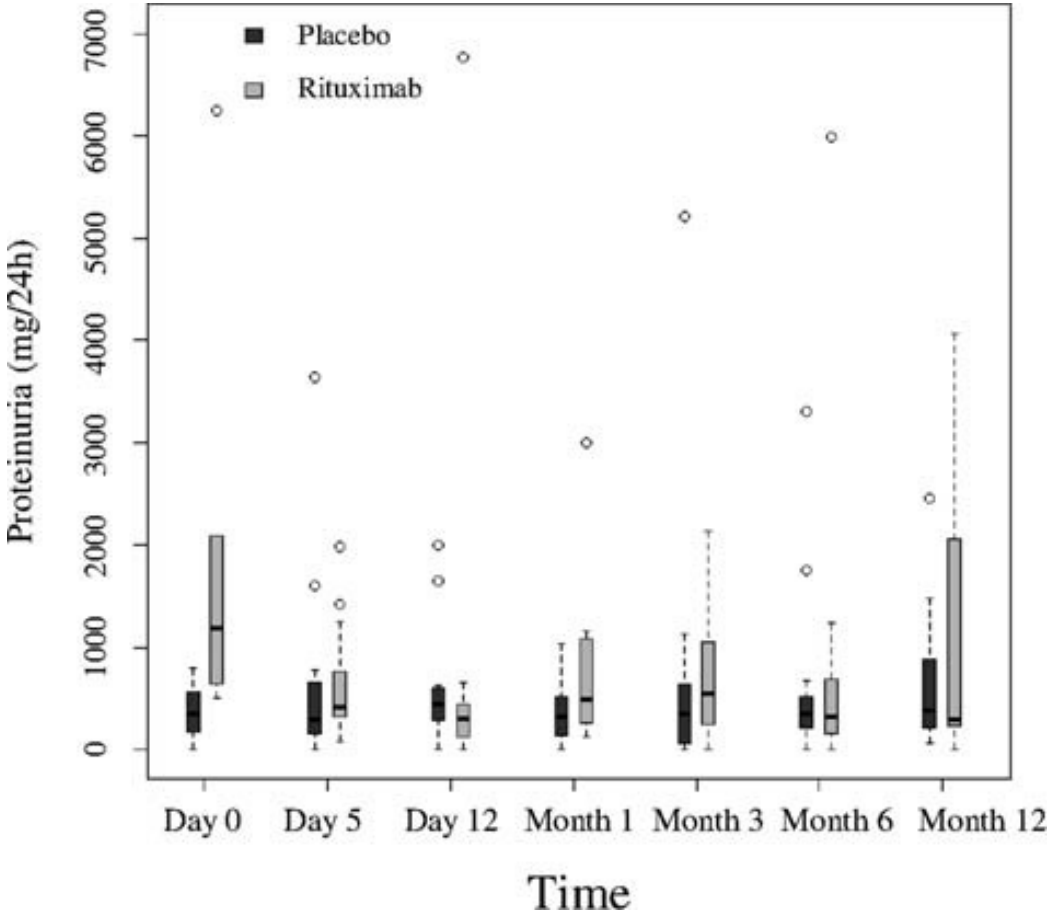
ALL: PE/3 CS pulses +

maintenance: steroids + tacrolimus (TL 8-12 ng/mL) + MMF (2 g/day)



Transplantation 2016;100: 391–399





Transplantation 2016;100: 391–399

Rituximab: infections

- none of the studies found a statistically significant higher incidence infectious of complications with rituximab. Indeed, significantly lower rates of CMV viremia and viral infections were identified, possibly for a lower number of episodes of rejection and associated steroid therapy (Transplantation 2014;98: 794-805)
- other reports suggest that desensitization with rituximab and IVIg may result in a greater incidence of BKV viremia after transplantation (Am J Transplant 2009; 9: 244, Transplantation 2014;97: 755-761)

Alemtuzumab

Lymphocyte-depleting, CD52-specific, monoclonal antibody: conflicting results

Alemtuzumab

- Potential negative effects of alemtuzumab on the regulation of humoral immunity, possibly due to dysregulation of B cell activating factor (BAFF), as an increase in BAFF mRNA expression include:
- unexpectedly high rates of ABMR
- high rates of circulating alloantibody
- intragraft C4d at 1-year posttransplant

Am J Transpl 2014; 14: 255–271

bortezomib

- Bortezomib is a proteasome inhibitor that acts on plasma cells and is effective in removing preformed DSA when combined with plasmapheresis
- It is also associated with durable reductions in DSA and stable allograft function in de novo DSA-positive renal transplant recipients

Am J Transpl 2014; 14: 255–271

bortezomib

- Prospective iterative trial:
- 44 sensitized patients treated – 19 transplanted
- median follow-up of 436 days
- acute rejection rates: 18.8%
- de novo DSA formation (12.5%).
- Patient and graft survival were 100% and 94.7%

Am J Transpl 2015; 15: 101–118

eculizumab

- 26 hyperimmune patients were treated with eculizumab post-transplantation vs 51 historical controls
- Both groups were treated pretransplantation with plasmaexchange (PE)
- After transplantation only control patients were treated by means of PE

Am J Transpl 2011; 11: 2405–2413

Results

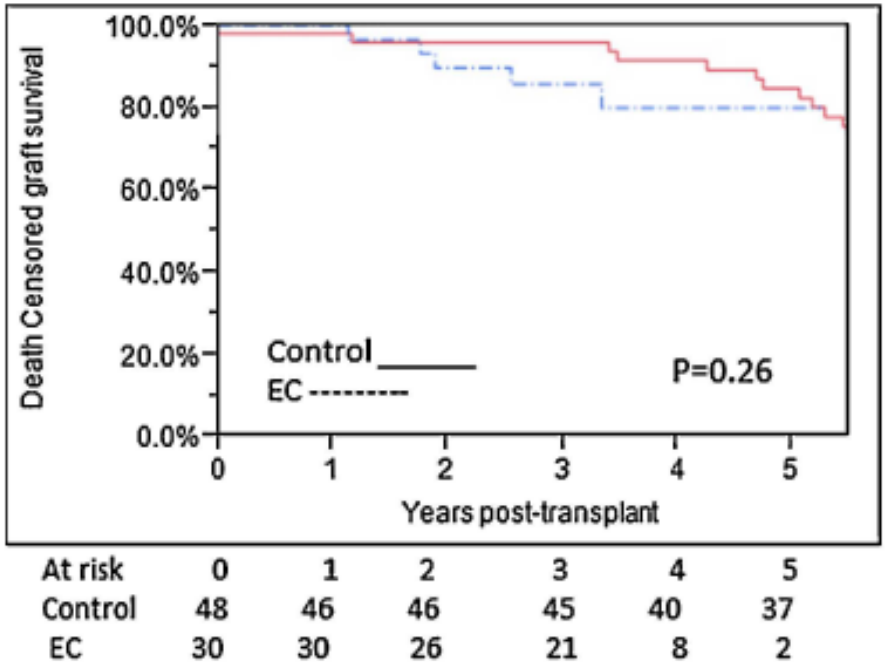
- incidence of AMR was 7.7% (2/26) in the eculizumab group compared to 41.2% (21/51) in the control group (p = 0.0031)
- On 1-year protocol biopsy, transplant glomerulopathywas found to be present in 6.7% (1/15) eculizumab-treated recipients and in 35.7% (15/42) of control patients (p = 0.044)

CONCLUSION: eculizumab decreases the incidence of early AMR in sensitized renal transplant recipients

Am J Transpl 2011; 11: 2405–2413

BUT … long term Results

A.



B.

	Eculizumab n=6	Control n=17	p-value
Transplant glomerulopathy	5(83.3%)	10 (58.8%)	P=0.37
Death with Function	1 (16.7%)	3 (17.6%)	P=1.0
Recurrent Focal Segmental Glomerulosclerosis	0 (0%)	1 (5.9%)	P=1.0
Recurrent IgA Nephropathy	0 (0%)	1 (5.9%)	P=1.0
Late Combined Cellular & Antibody Mediated Rejection	0 (0%)	1 (5.9%)	P=1.0
Unknown	0 (0%)	1 (5.9%)	P=1.0

CONCLUSION: despite decreasing acute clinical ABMR rates, EC treatment does not prevent chronic ABMR in recipients with persistently high BFXM after XMKTx.

Am J Transplant. 2015;15:1293-302

C1 Inhibition

- C1 inhibitor (C1-INH) is a multifunctional member of the serpin family of protease inhibitors. C1-INH inactivates both C1r and C1s and is the only plasma protease that regulates the classic complement pathway
- All patients with PRA > 50 % treated with rituximab + IgG
- 10 pts treatd with C1-INH and 10 with placebo
- Primary end point: ABMR at 6 months

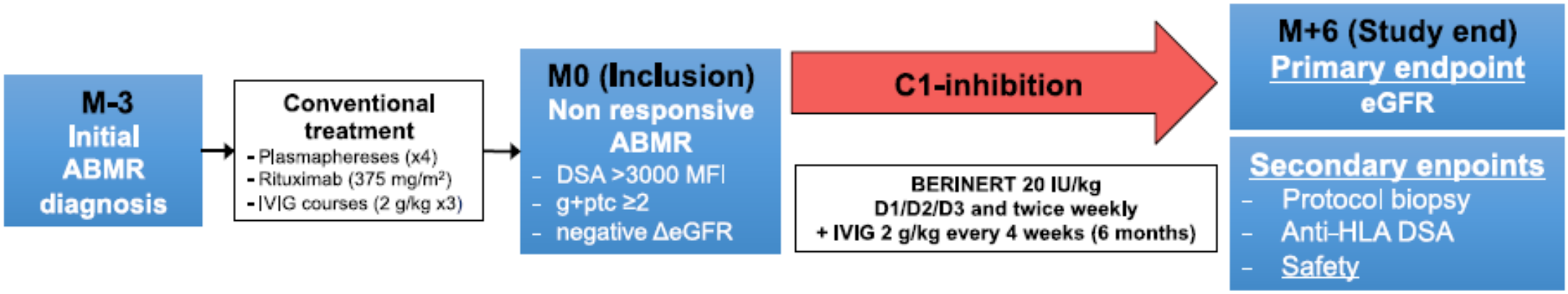
Transplantation 2015;99: 299–308

C1 Inhibition: results

- No significant difference was seen in rejection rate between treated and non treated patients
- in vitro experiments revealed that C1-INH was very efficient at inhibiting C1q binding to luminex beads induced by low-titer HLA antibodies and less effective with high-titer antibodies

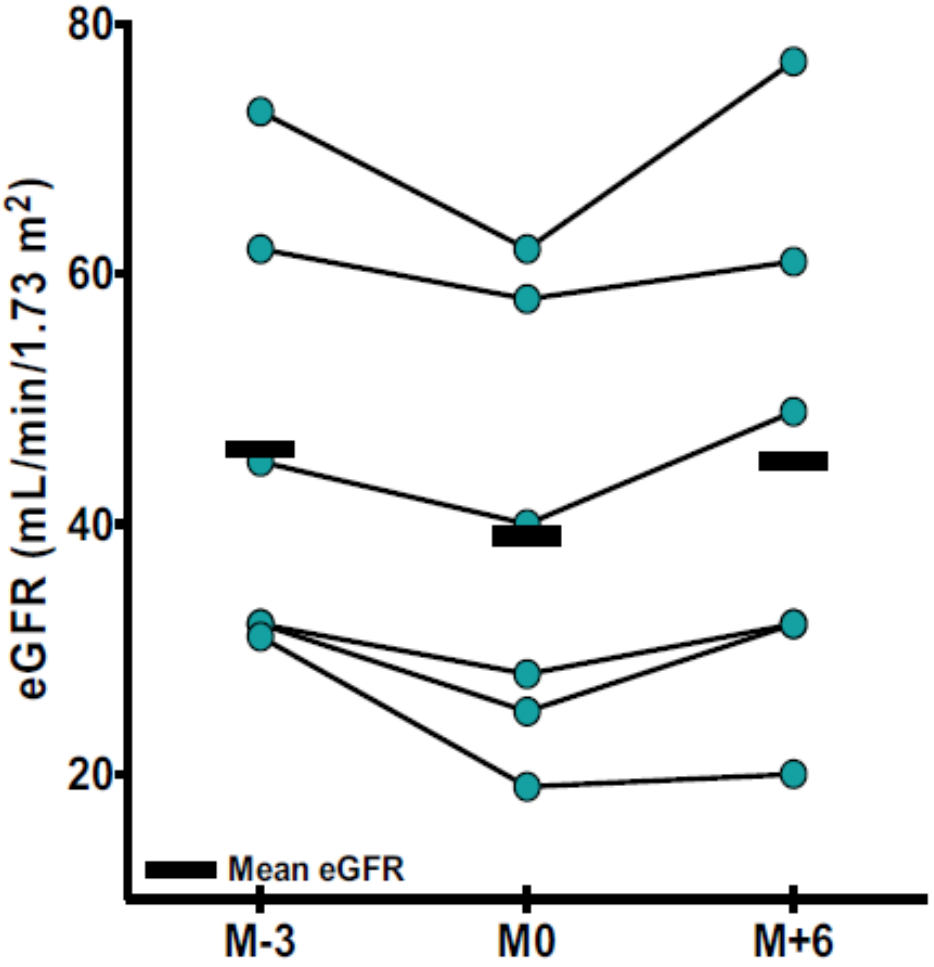
Transplantation 2015;99: 299–308

C1 Inhibition



- 6 patients were treated

Am J Transplant. 2016;16(5):1596-603



Am J Transplant. 2016;16(5):1596-603

Sensitized patients

DSA removal (immunoadsorption or plasma exchange), DSA inactivation (high-dose intravenous immunoglobulins) enable successful positive-crossmatch kidney transplantation with good short- to intermediate term outcomes

Nat. Rev. Nephrol. 6, 297–306 (2010);

However:

Antibody-mediated rejection can occur subclinically and in time results in chronic injury to the renal microvasculature, transplant glomerulopathy, interstitial fibrosis, and tubular atrophy

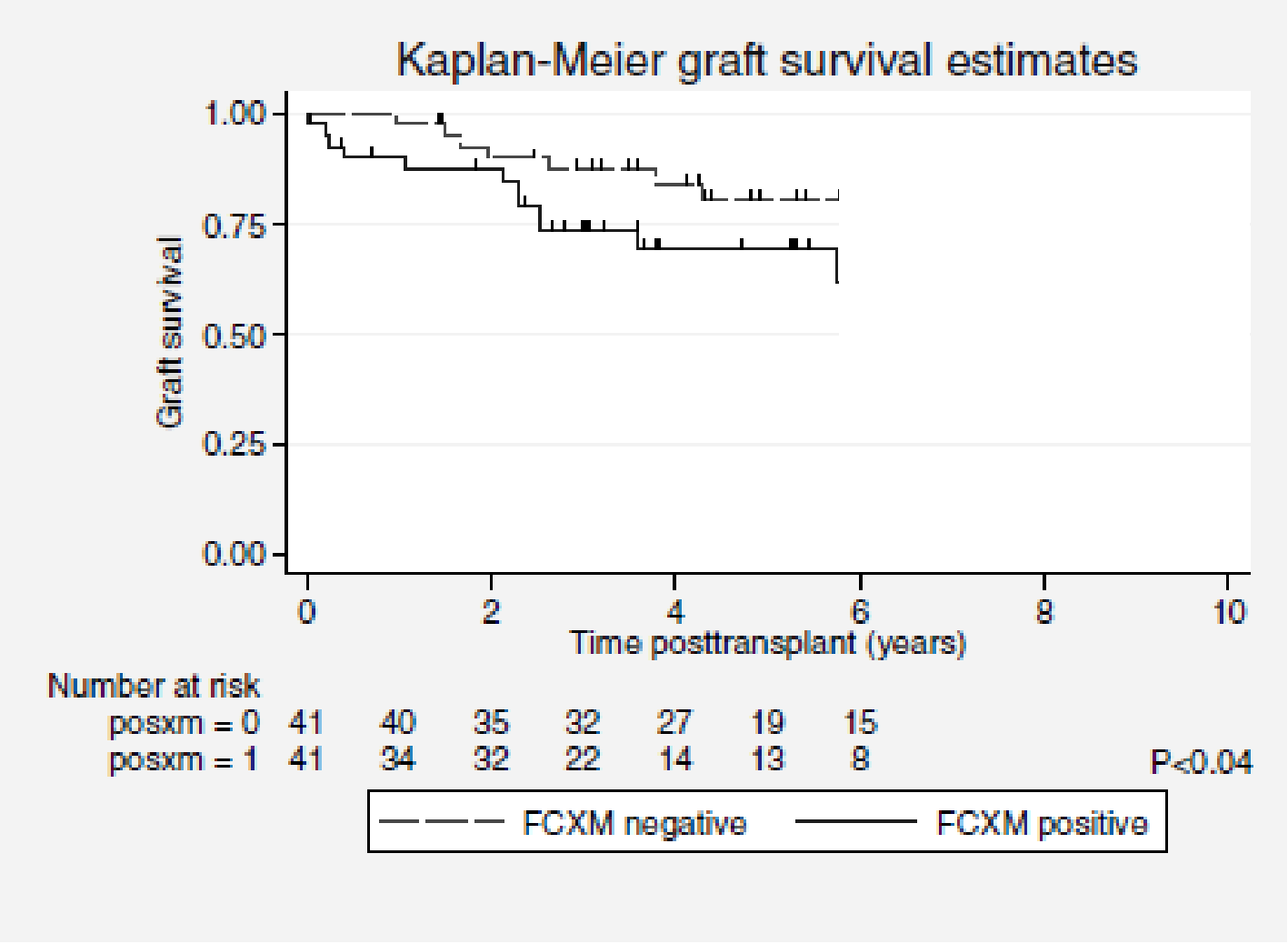
Nat. Rev. Nephrol. 6, 297–306 (2010);

and

- acute antibody mediated rejection (AMR) occurs in 20–50% of positive crossmatch transplantations.
- AMR is usually reversed:1 year survival close to 90%
- but 3, 5 or 8 years survival significantly worse than “standard”

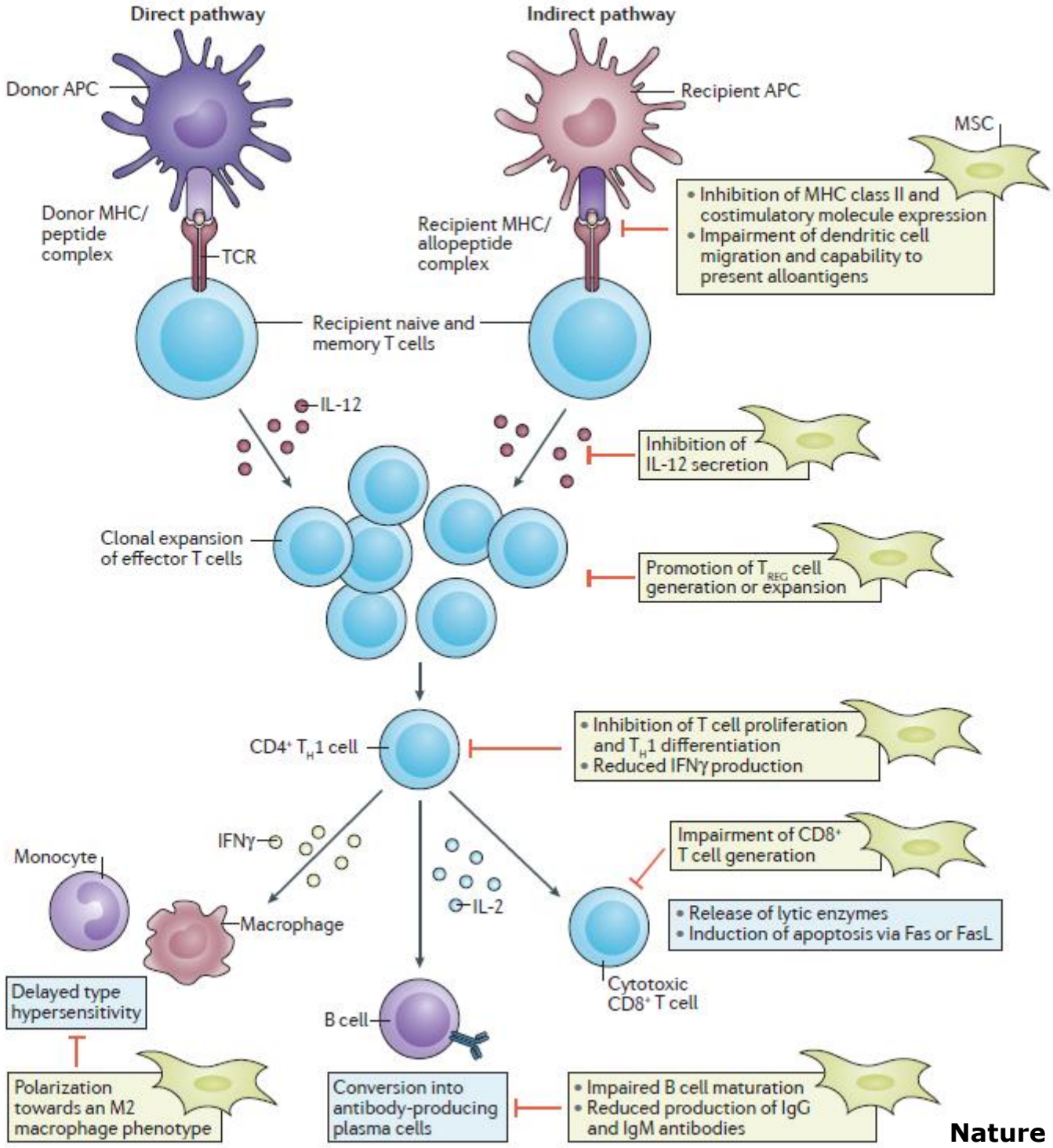
Nat. Rev. Nephrol. 6, 297–306 (2010);

Graft survival in positive cross-match cases compared with controls



Am J Transpl 2009; 9: 536–542

IS THERE (ALREADY) A ROLE FOR MESENCHIMAL STEM CELLS?



Nature Rev Nephrol: 2016; 12:243

Table 2 | Clinical studies of BM-derived MSCs in kidney transplantation

Study	Induction therapy (dose)	Maintenance immunosuppression	No. of patients	MSC			Main finding
				Source	Dose (cells per kg $\times 10^6$)	Timing	
Perico *et al.* (2011)[99]	rATG (0.5 mg, day 0–6); basiliximab (20 mg days 0 and 4); steroids (day 0 to 7)	CsA, MMF	2	Autologous	1.7–2.0	Day 7	Increased T_{REG} cell:memory CD8 T cell ratio from baseline; engraftment syndrome in two patients
Perico *et al.* (2013)[100]	rATG (0.5 mg, day 0–6); steroids (day 0–7)	CsA, MMF	2	Autologous	2.0	Day -1	Increased T_{REG} cell:memory CD8 T cell ratio from baseline; acute cellular rejection in one patient
Tan *et al.* (2012)[101]	Basiliximab in control group only (20 mg, days 0 and 4)	CNI, MMF, steroids	105 (53 on standard CNI dose; 52 on 80% CNI dose)	Autologous	1.0–2.0	Day 0 and 14	Reduced incidence of acute rejection at 6 months and lower incidence of viral infections in the MSC group than in the control group
Reinders *et al.* (2013)[102]	Basiliximab (20 mg, day 0 and 4)	CNI, MMF, steroids	6	Autologous	1.0–2.0 (two doses 7 days apart)	Week 4 or month 6	MSC infusion enabled resolution of tubulitis and IFTA in two patients with subclinical rejection; opportunistic viral infection in three patients
Mudrabettu *et al.* (2015)[103]	rATG (1mg/kg, day -1 to +1)	Tacrolimus, MMF, steroids	4	Autologous	0.2–0.8	Day -1 and 30	No early or late kidney graft dysfunction and no viral infections in the KTRs
Peng *et al.* (2013)[104]	Cytoxan (200 mg)	Tacrolimus, MMF, steroids	6	Donor	5.0 (renal artery at day 0) and 0.2 (IV day 30)	Day 0 and 30	50% reduction of tacrolimus dose in the MSC group

BM, bone marrow; CNI, calcineurin inhibitor (CsA or tacrolimus); CsA, ciclosporin A; IFTA, interstitial fibrosis and tubular atrophy; IV, intravenous; KTR, kidney transplant recipient; MMF, mycophenolate mofetil; MSC, mesenchymal stromal cell; rATG, rabbit anti-thymocyte globulin; T_{REG}, T regulatory.

Nature Rev Nephrol: 2016; 12:243

Table 1 Registered clinical trials of mesenchymal stem cells in kidney transplantation (ClinicalTrial.gov, updated July 2015)

NCT	Status	Title	Site	Type of MSC	Start date
NCT02409940	Recruiting	To elucidate the effect of mesenchymal stem cells on the T-cell repertoire of kidney transplant patients	Chandigarh, India	Autologous/allogeneic; BM-MSC	September 2013
NCT02387151	Recruiting	Allogeneic mesenchymal stromal cell therapy in renal transplant recipients	Leiden, Netherlands	Allogeneic; BM-MSC	March 2015
NCT02057965	Recruiting	Mesenchymal stromal cell therapy in renal recipients	Leiden, Netherlands	Autologous; BM-MSC	March 2014
NCT02012153	Recruiting	Mesenchymal stromal cells in kidney transplant recipients	Bergamo, Italy	Autologous; BM-MSC	December 2013
NCT00659620	Unknown	Mesenchymal stem cell transplantation in the treatment of chronic allograft nephropathy	Fuzhou, Fujian	Autologous; BM-MSC	May 2008
NCT00734396	Completed	Mesenchymal stem cells and subclinical rejection	Leiden, Netherlands	Autologous; BM-MSC	February 2009
NCT00752479	Terminated	Mesenchymal stem cells under basiliximab/low dose RATG to induce renal transplant tolerance	Bergamo, Italy	Autologous; BM-MSC	May 2008
NCT00658073	Completed	Induction therapy with autologous mesenchymal stem cells for kidney allografts	Fuzhou, Fujian	Autologous; BM-MSC	March 2008
NCT01429038	Recruiting	Mesenchymal stem cells after renal or liver transplantation	Liege, Belgium	Allogeneic; BM-MSC	February 2012

BM-MSC bone marrow-derived mesenchymal stem cell, *MSC* mesenchymal stem cell, *NCT* ClinicalTrials.gov identifier, *RATG* rabbit antithymocyte globulin

Non-HLA antibodies

Pharmacologic antagonists targeting the ETAR (sentanes)??

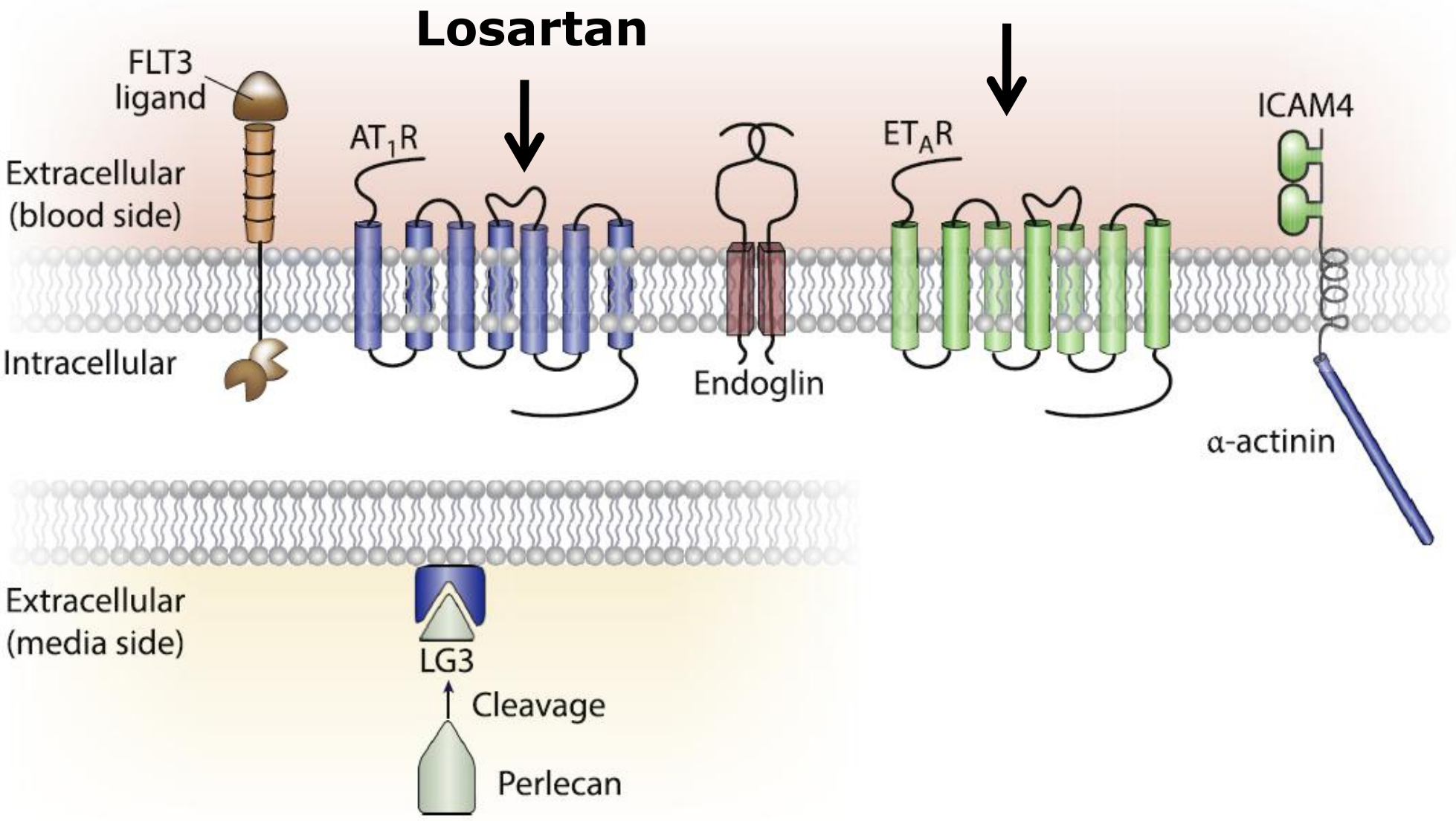


Figure 1 | Overview of nonhuman leukocyte antigen antibodies directed against endothelial targets. AT_1R, angiotensin II type 1 receptor; ET_AR, endothelin-1 type A receptor; FLT3, Fms-like tyrosine kinase-3; ICAM4, intercellular adhesion molecule 4.

Kidney Int. 2016; 90:280-8

Conclusions I

- Donor Specific Antibodies worsen graft outcome
- They may be directed toward several different antigens

Conclusions II

- Current therapies, including
  - DSA removal (plasma exchange/immunoadsorption)
  - DSA modulation (intravenous immunoglobulin ± rituximab)
  - complement component antagonists (eculizumab)

  have been relatively successful to treat **acute** AMR.
- In contrast, chronic progression in AMR has proven to be intractable so far

Conclusions III

early identification of non HLA antibodies could lead to timely initiation of possibly effective targeted therapies