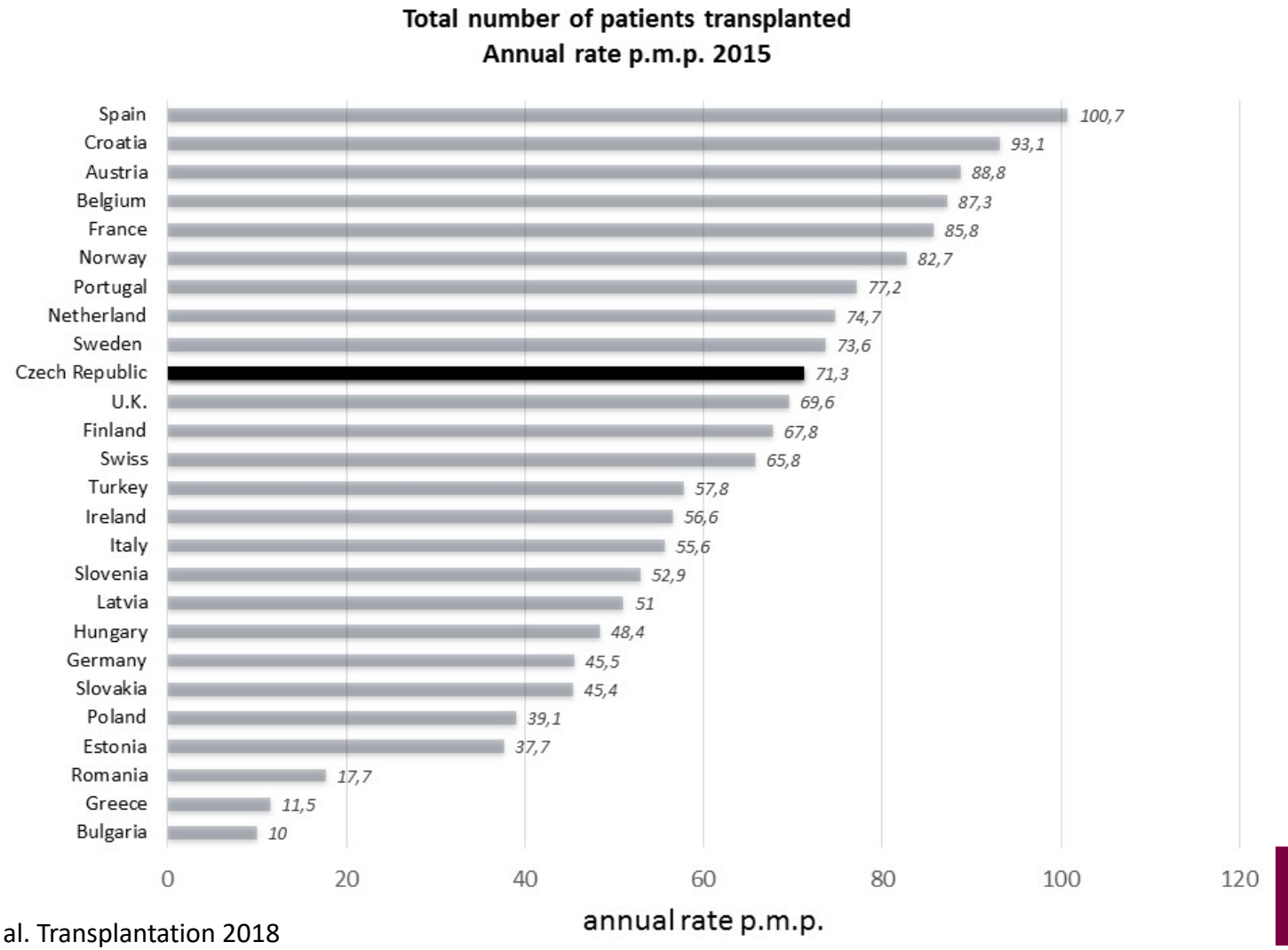
Imunologické vyšetřování nemocných před a po transplantaci ledviny

Ondřej Viklický

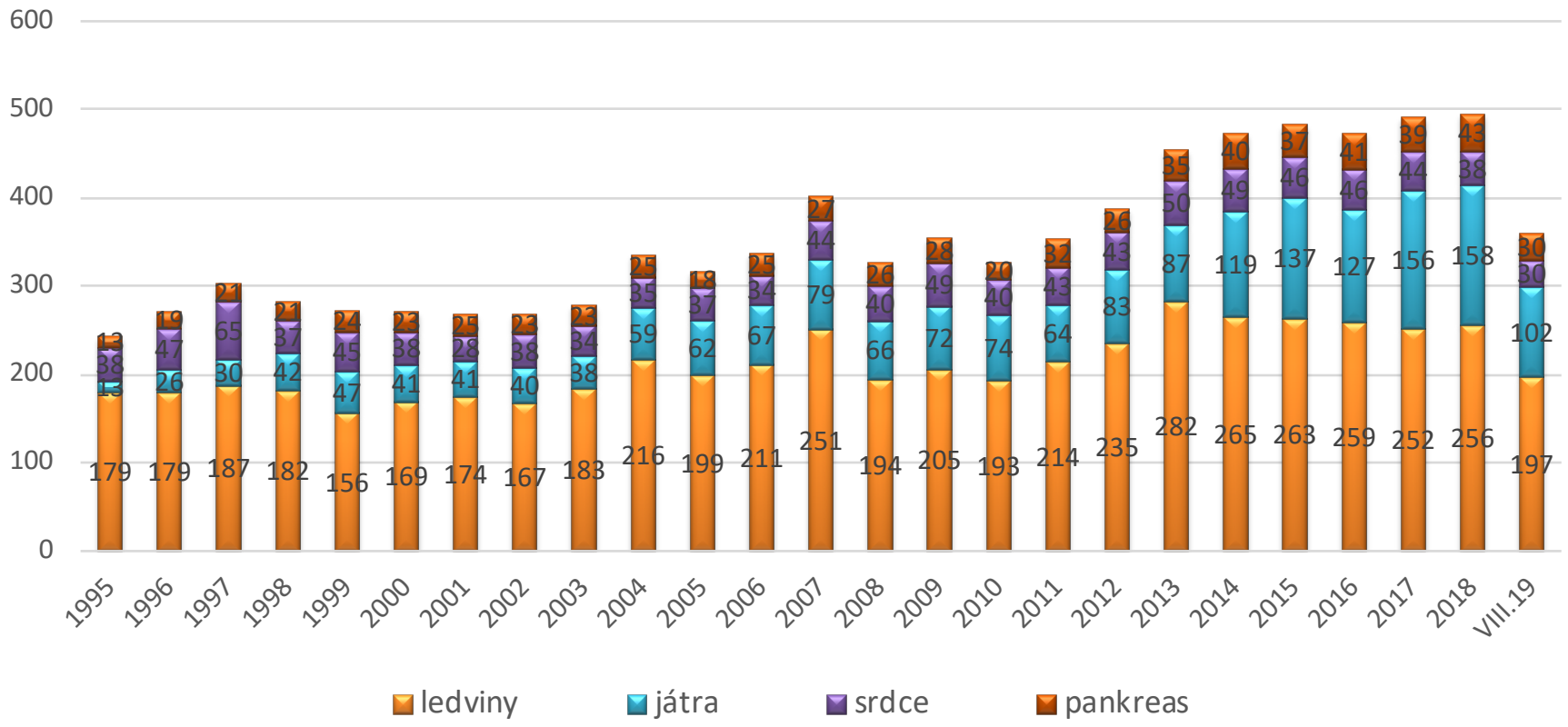
Klinika nefrologie IKEM, Praha

Dostupnost transplantací je v ČR na vysoké úrovni

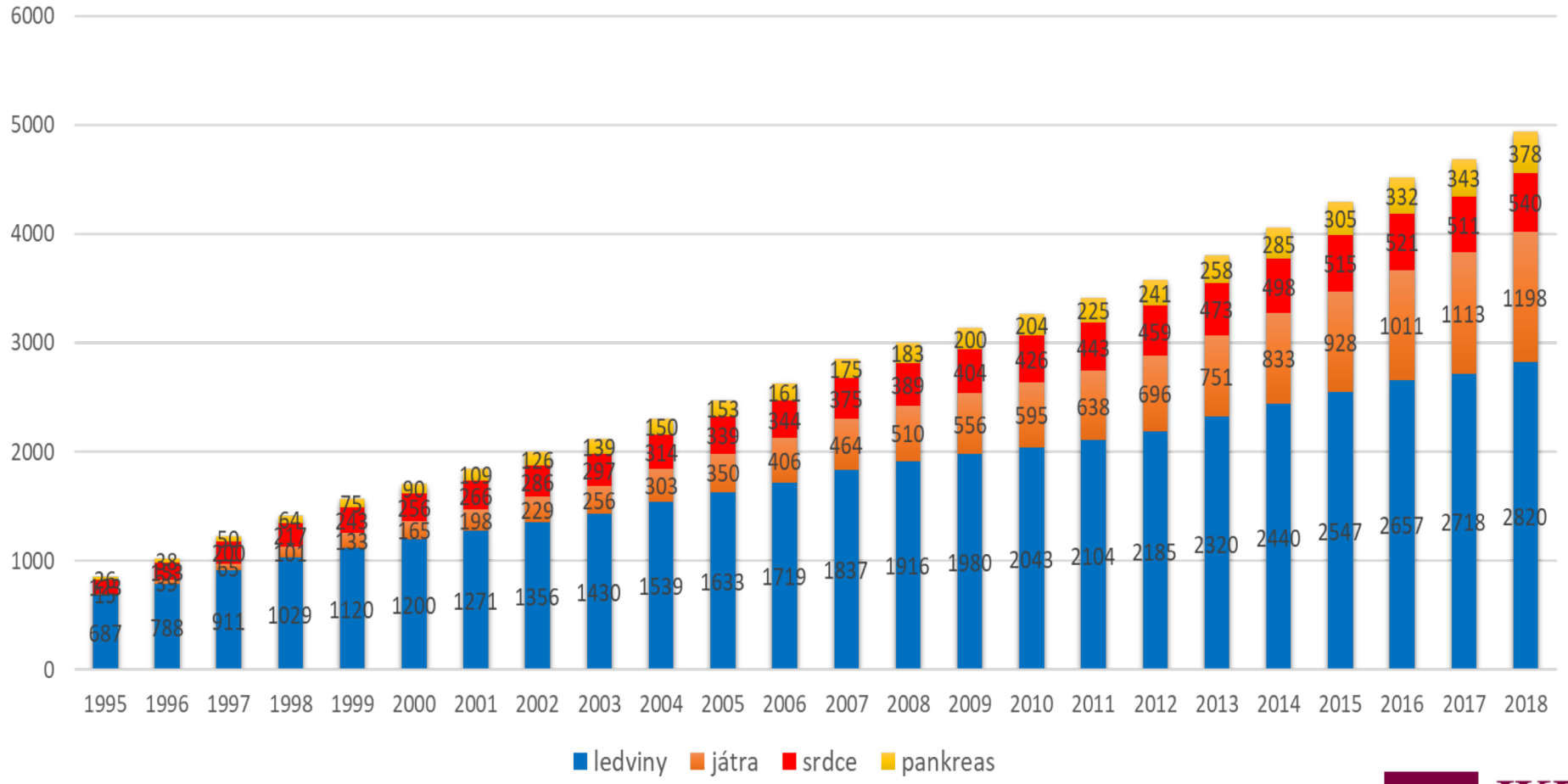


Viklicky O. et al. Transplantation 2018

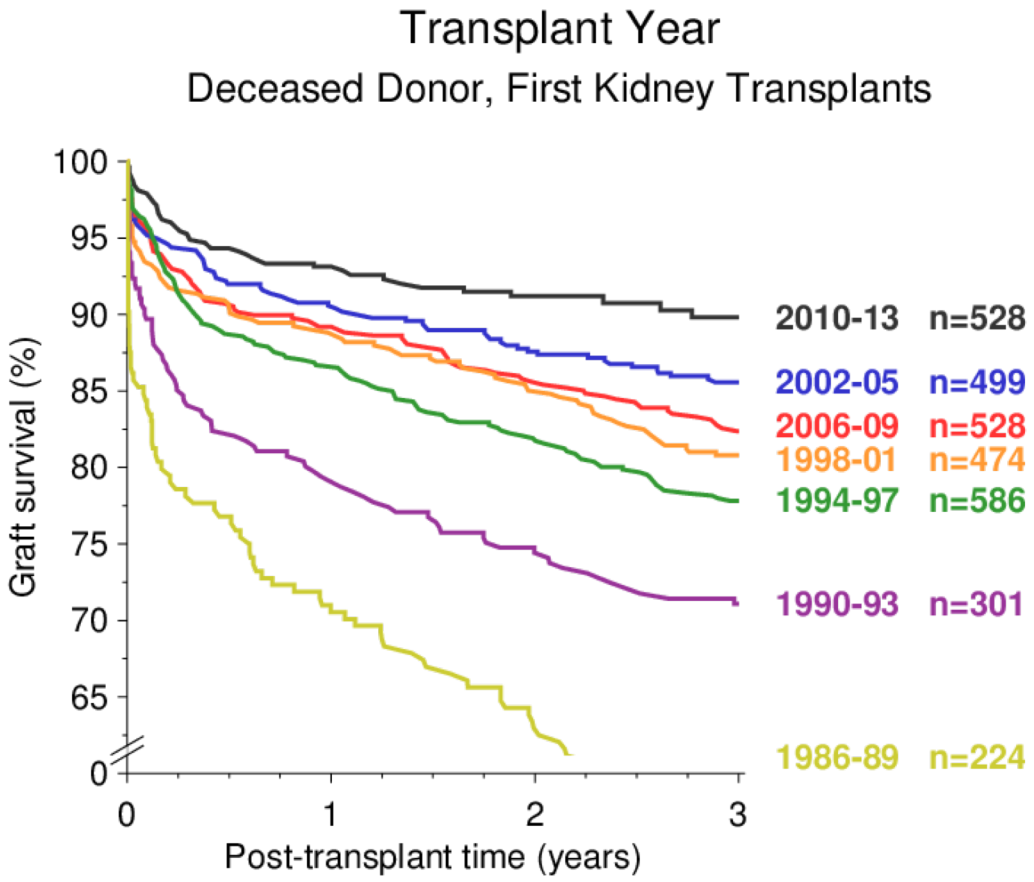
Transplantace orgánů v TC IKEM (1995-8/2019)



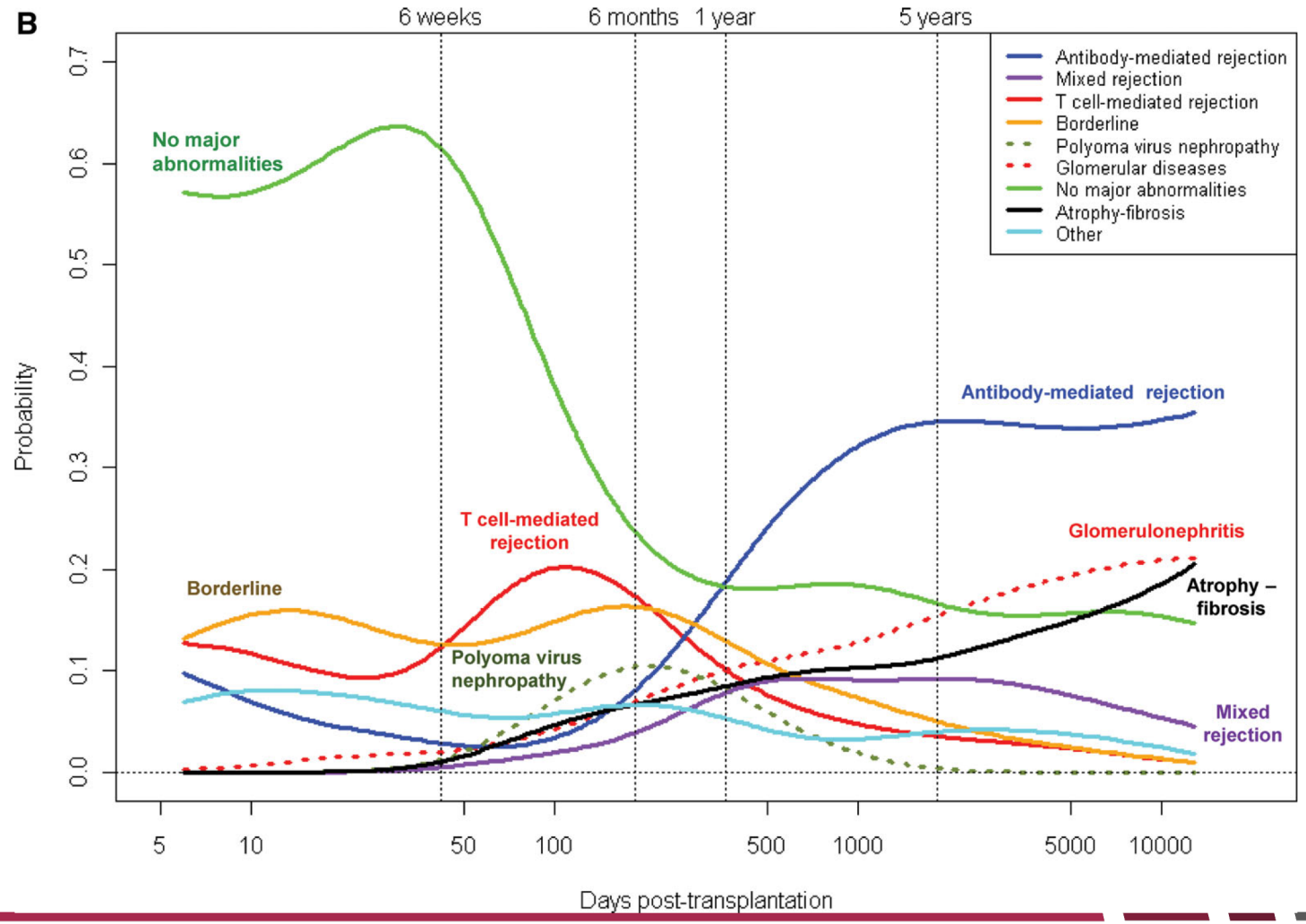
Nemocní žijící s funkčními transplantovanými orgány v IKEM



Zlepšení výsledků transplantací ledvin



Výskyt patologií štěpu po transplantaci



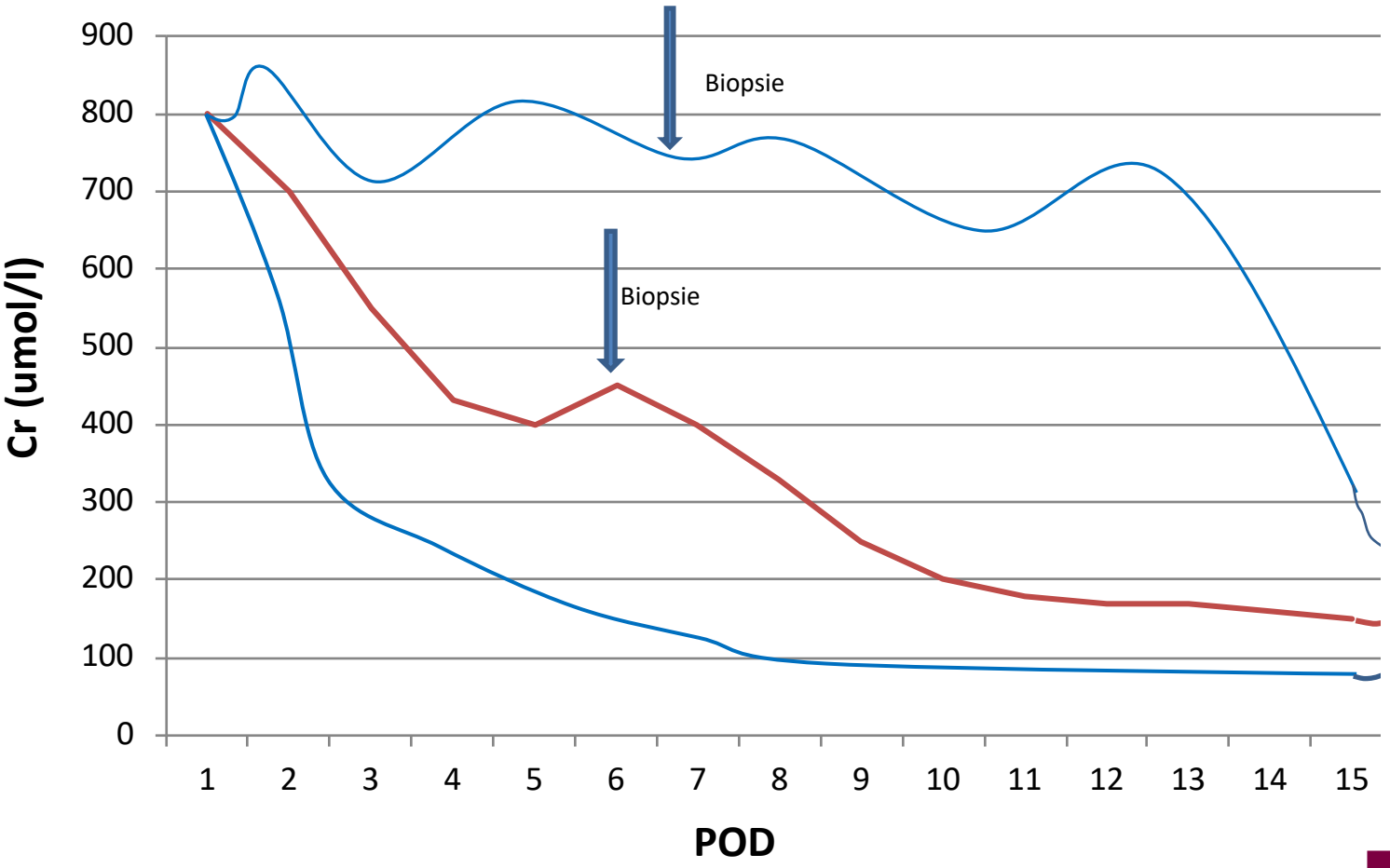
Sellares J. et al. Am J Transplant 2011, doi: 10.1111/j.1600-6143.2011.03840.x

Vyšetření imunitní odpovědi

- Před transplantací
- Po transplantaci

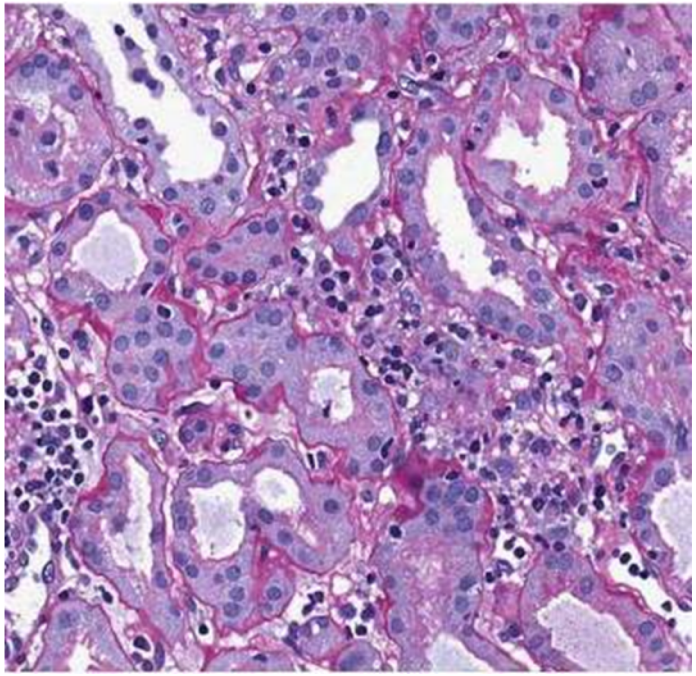
- Invazivní – protokolární biopsie
- Neinvazivní – z krve/moče

Rejekce nebo ATN?



Borderline changes

Banff´s classification
histological changes insufficient for a diagnosis of acute rejection.
Absence of intimal arteritis and the presence of tubulitis (t1,t2 or t3) with minor intersticial infiltration ((i0, i1) or intersticial infiltration(i2, i3) with mild tubulitis (t1)



- **Incidence** : in case biopsies 23% of renal allografts

Meehan et al. (1999) J Am Soc Nephrol 10: 1806
Matoza et al. (2008) Transplantation Proceedings 40: 2303-2306
Viklický et al. (2013) Transplantation 95: 148-154

- The outcome of borderline changes unclear

- The majority of patients with borderline changes were found to be non-rejection by molecular phenotype(67%) and only 33% were rejection-like.

de Freitas et al. (2012) American Journal of Transplantation. 12: 191–201

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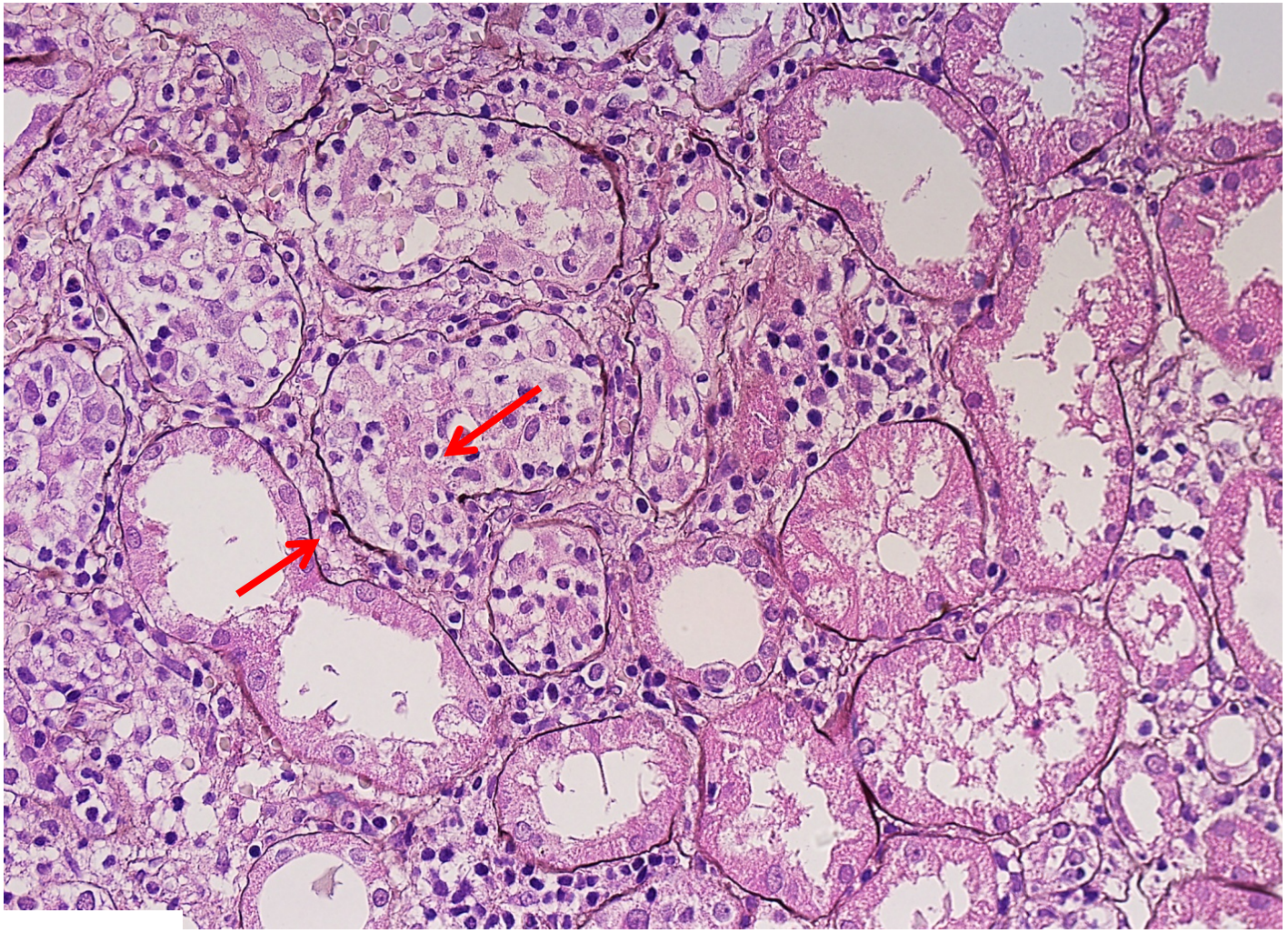
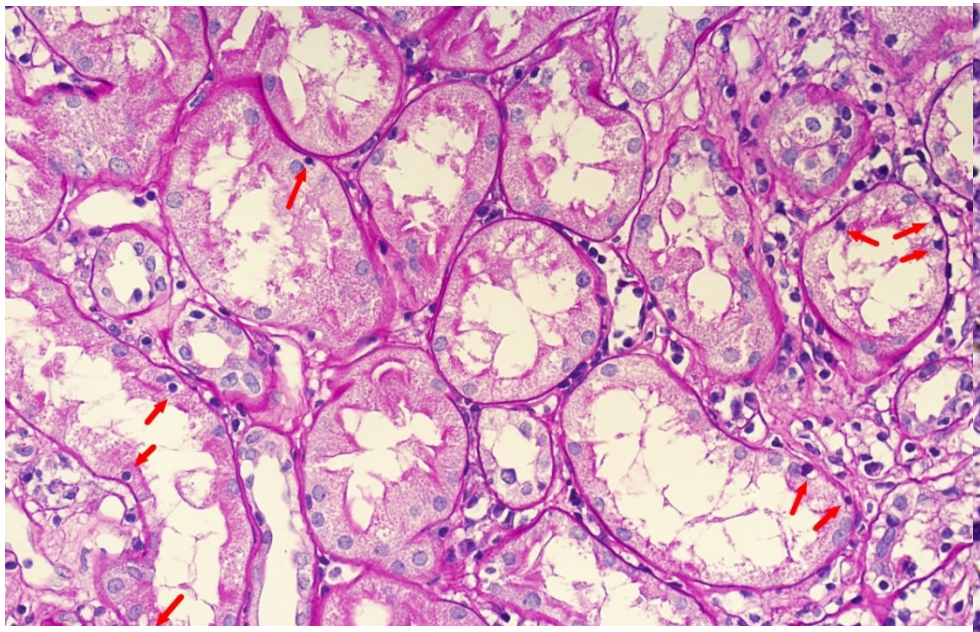
Tubulitis

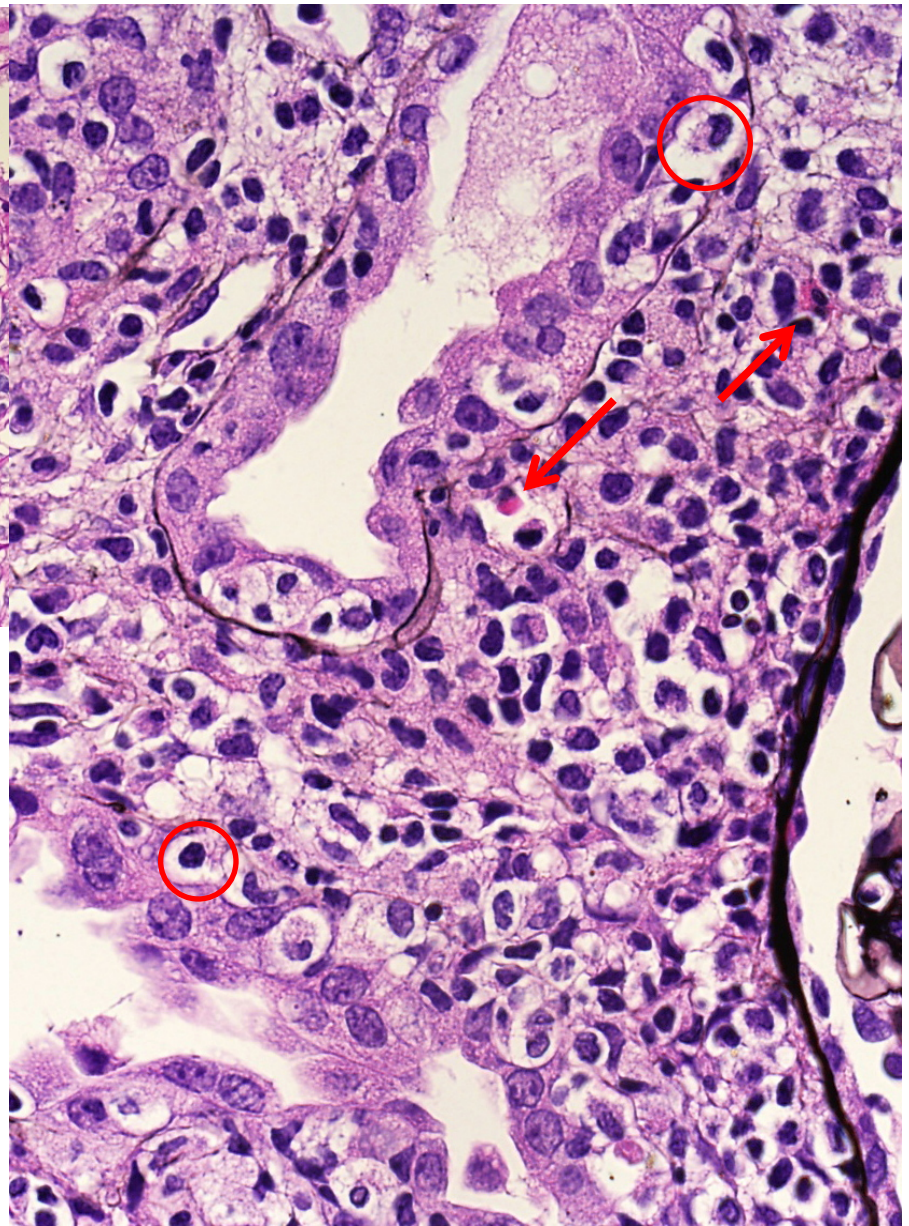


E. Honsová; IKEM

Acute T-cell-mediated rejection, type I

tubulitis in non-atrophic tubules





Banff criteria:

- **IA. i:>25% of parenchyma affected, i2 or i3 and t2**
- **IB. i> 25% of parenchyma affected, i2 or i3 and t3**

Chapel Hill Standards

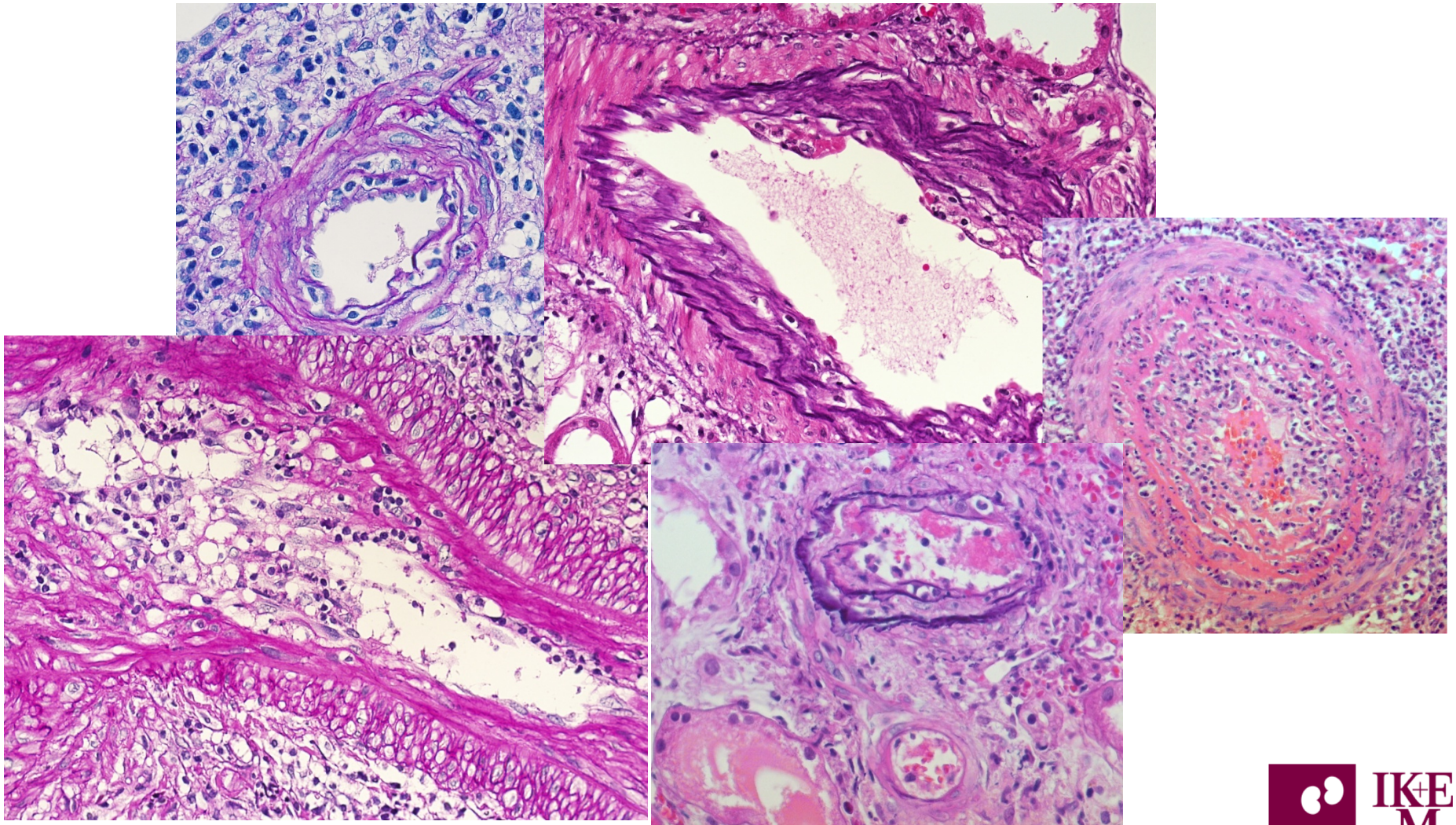
1. i ≥5%
2. Mild to moderate interstitial edema
3. Tubulitis (t1 in ≥3 tubules in most inflamed area),

- scattered eosinophils and ATI are common
- MHC class II in tubular cells
- If criteria 1-3 are not fulfilled, but tubules strongly expressed MHC class II, then an episode of ACR is suggested

E. Honsová; IKEM

Acute T-cell-mediated rejection, type II and III

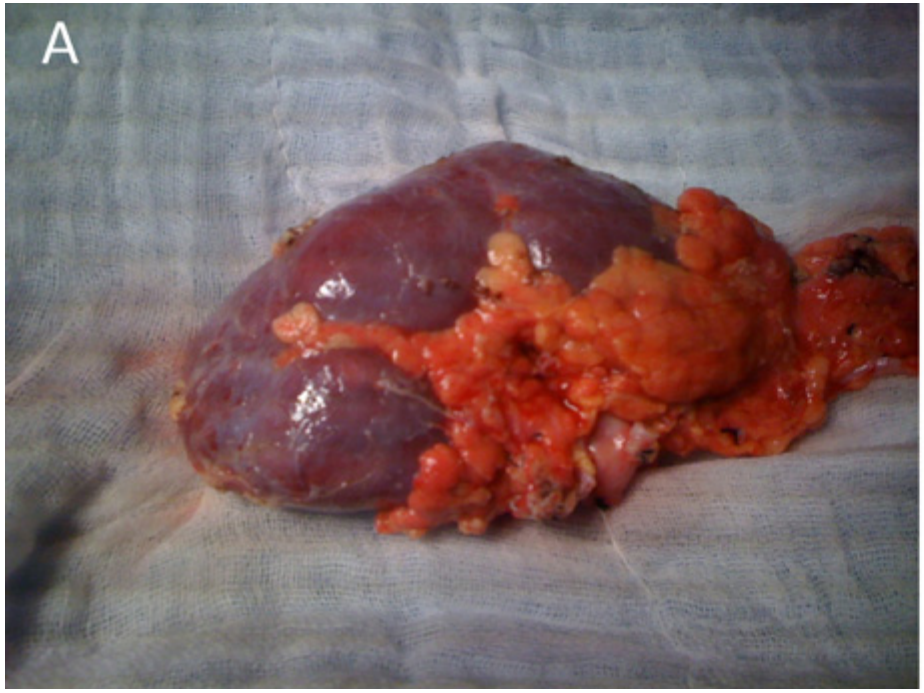
arteritis or endotheliitis

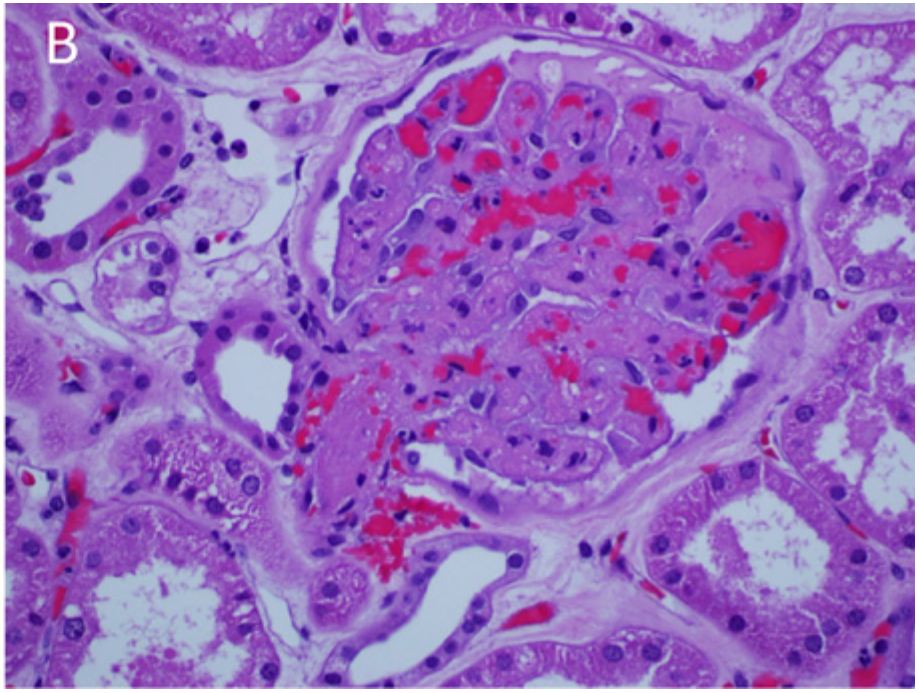


E. Honsová; IKEM

IK+E M

Makroskopické a mikroskopické projevy hyperakutní rejekce





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

Diagnostika akutní AMR 2002

- Diagnóza vyžaduje přítomnost 3 kritérií
  - I. Dysfunkce štěpu
  - II. Histologické známky AMR
    - C4d depozita v peritubulárních kapilárách (PTC)
    - Neutrofily v PTC a glomerulárních kapilárách
    - Tubulitis s neutrofily
    - Fibrinoidní nekrosy v arteriích a glomerulech
  - III. Detekovatelné dárcovsky specifické protilátky (DSA)

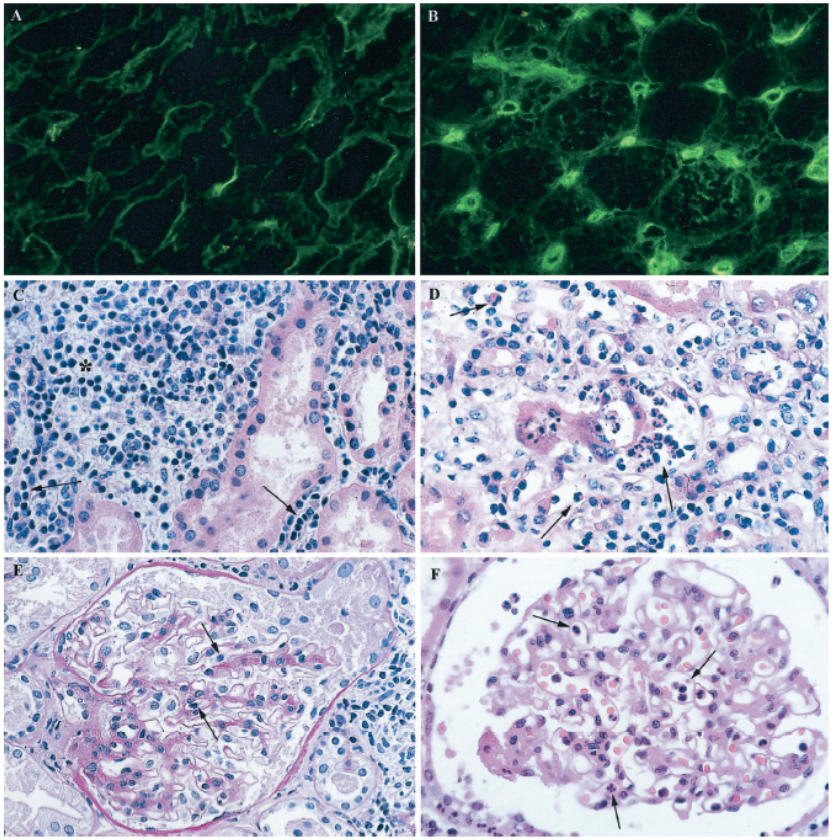


Figure 1. (A) Acute cellular rejection (ACR): no staining for C4d is seen in peritubular capillaries. (B) Acute humoral rejection (AHR): widespread and bright staining for C4d is present in the peritubular capillaries that are interspersed in between the silhouettes of tubules. (C) ACR: mononuclear cells are present in the interstitium (*) and in peritubular capillaries (arrows). (D) AHR: abundant neutrophils are present in dilated peritubular capillaries (arrows). (E) ACR: scattered mononuclear cells are present in glomerular capillaries (arrows). (F) AHR: neutrophils are present in glomerular capillaries (arrows). Staining: C4d-FITC in A and B; Hematoxylin and eosin (H&E) in C, D, and F; and periodic acid-Schiff (PAS) in E. Magnifications: ×400 in A through D; ×450 in E and F.

Racusen L, et al. Am J Transplant 2003;3:708.
Mauiyyedi S. et al. J Am Soc Nephrol 2002;13:779.

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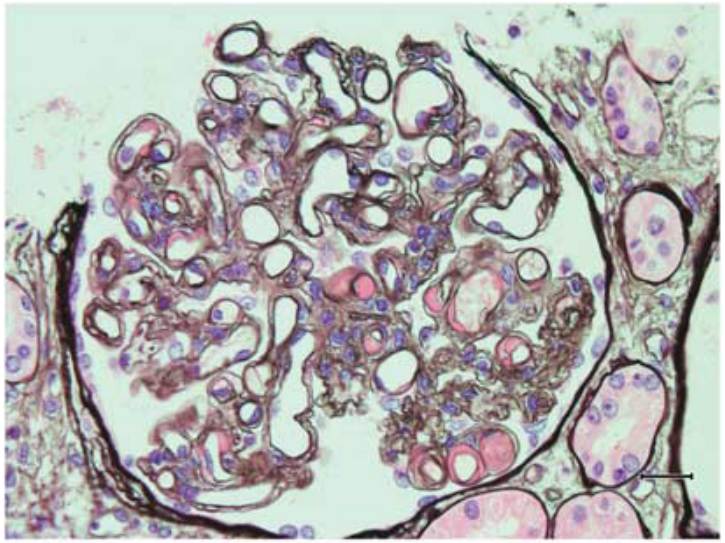
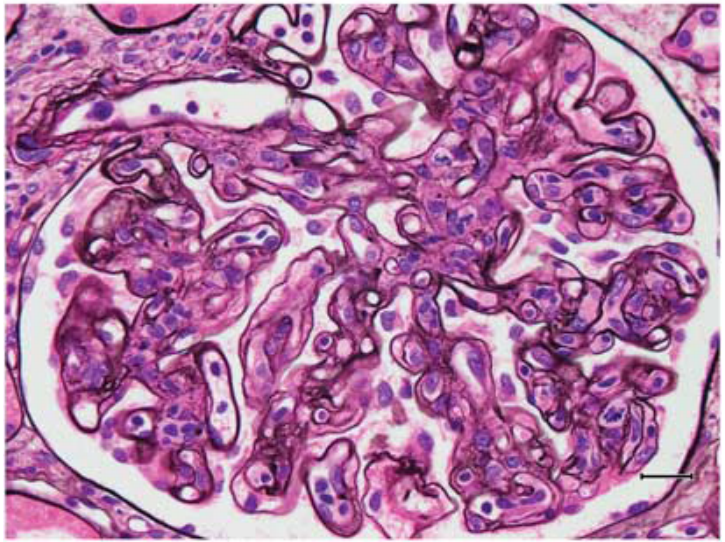
Transplantační glomerulopatie nespecifický obraz poškození?





- Protilátkami zprostředkovaná rejekce
- HCV
- Trombotická mikroangiopatie

- TG má nejhorší prognosu: 50% ztrát štěpů do 5 let

Haas. Kidney Int 2011; 80, 801 – 803.

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Klasifikace histologických nálezů transplantované ledviny

- Category 1: Normal/nonspecific changes
- Category 2:Antibody-mediated changes
  - Acute/active ABMR
  - Chronic active ABMR
  - C4d staining without evidence of rejection
- Category 3: Borderline changes
- Category 4:TCMR
  - Acute TCMR Grade: IA , IB, IIA, IIB, III
  - Chronic active TCMR
- Category 5: Interstitial fibrosis and tubular atrophy
- Category 6: Other changes not considered to be caused by acute or chronic rejection

The Banff 2015 Kidney Meeting Report, American Journal of Transplantation 2017; 17: 28–41

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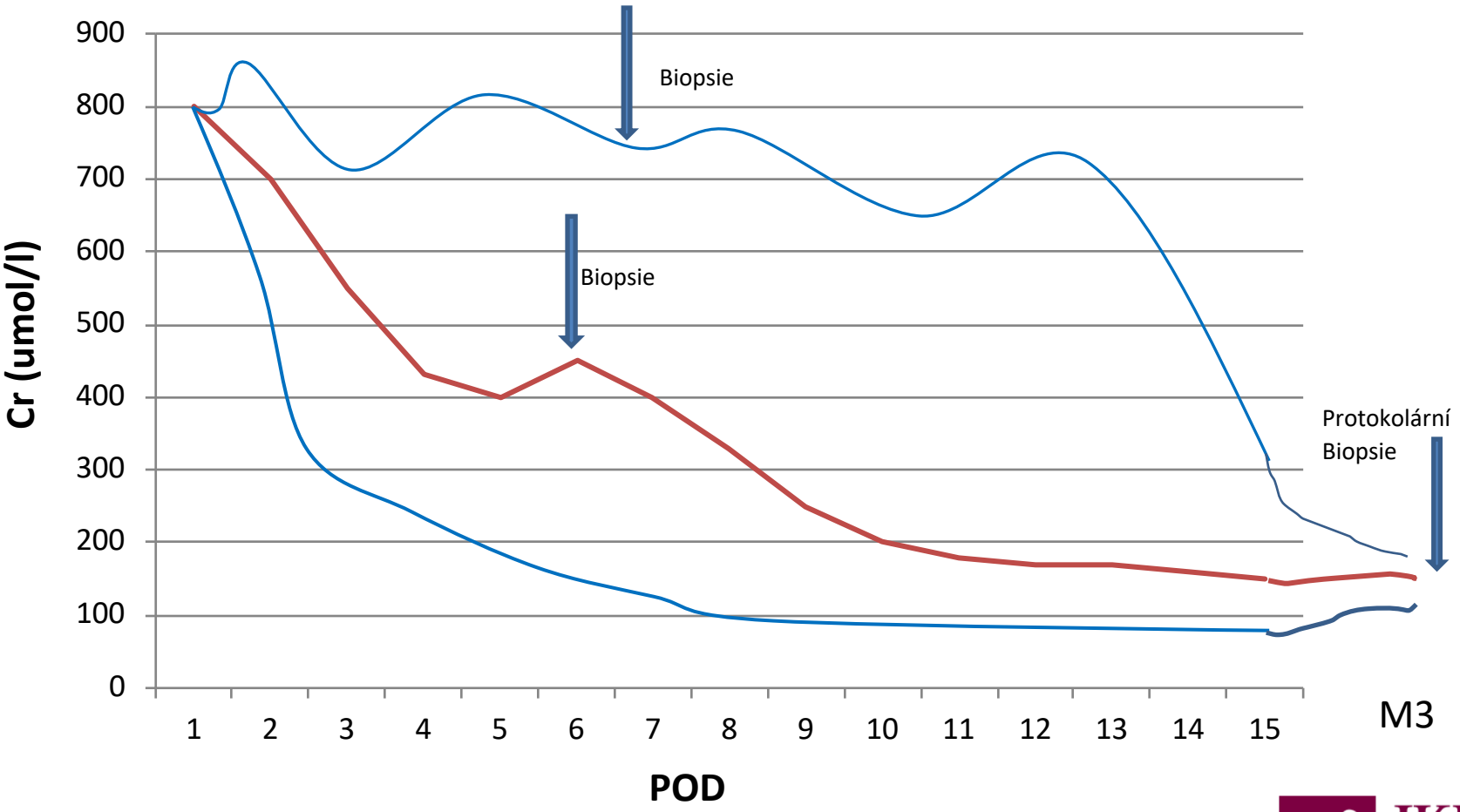
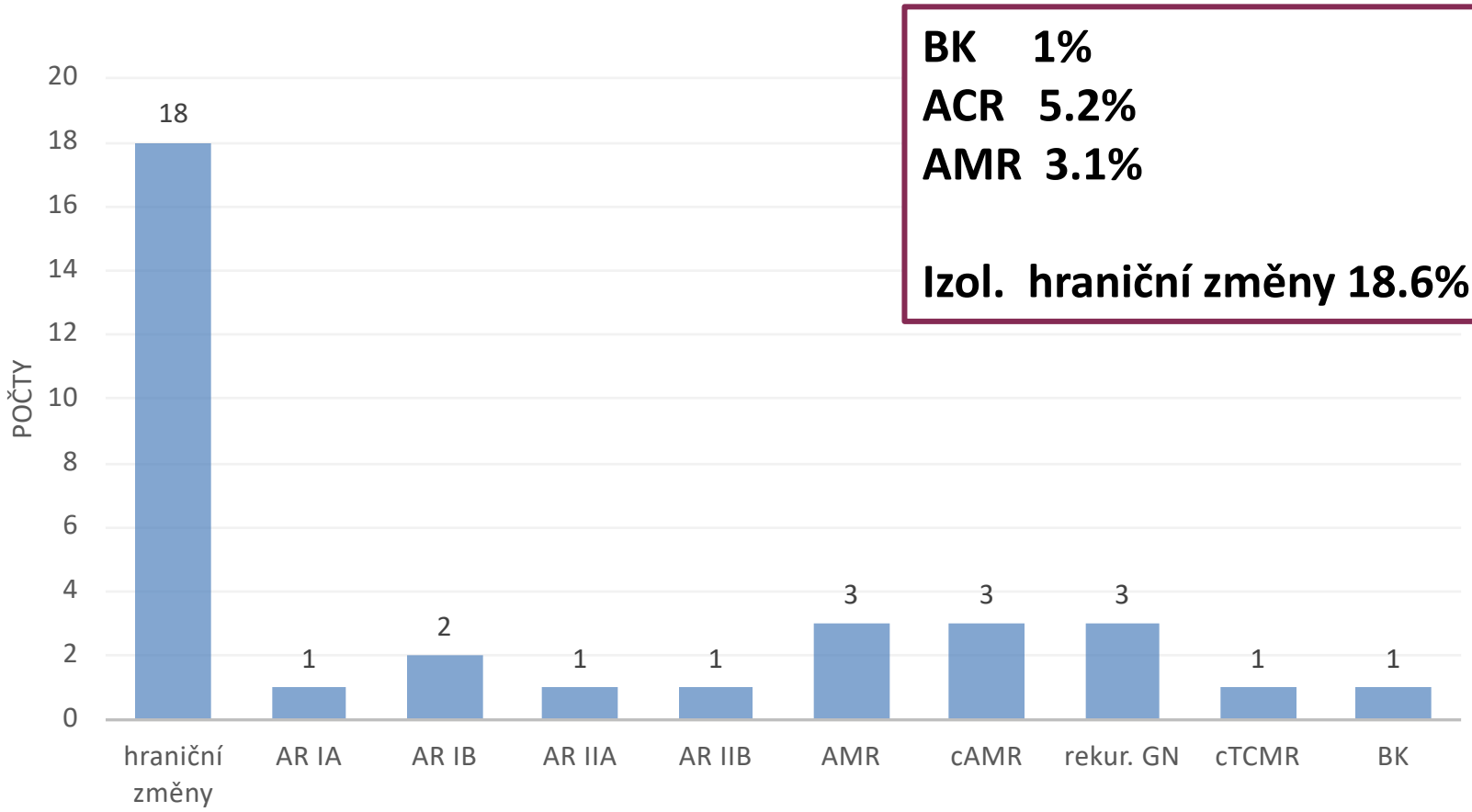
Rejekce nebo ATN?



Invazivní monitorace imunitní odpovědi: protokolární biopsie ve 3. měsíci

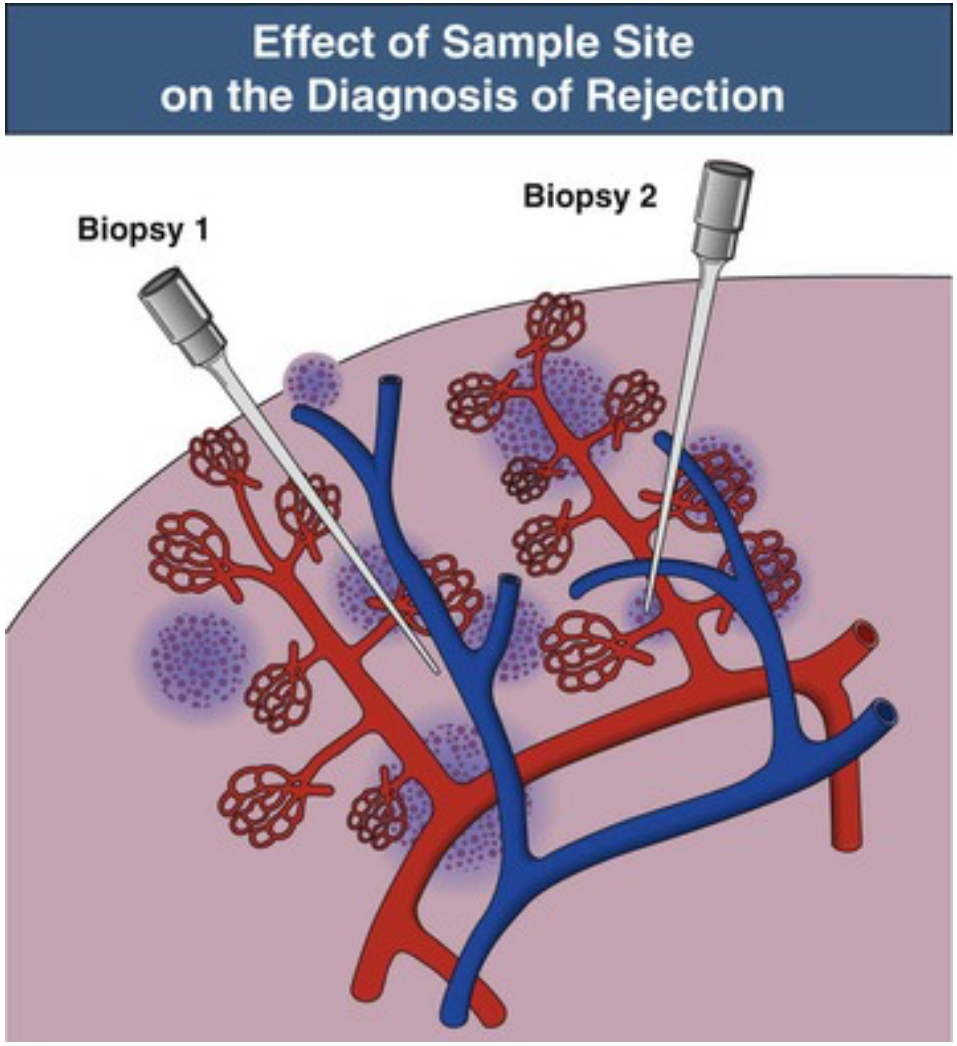
rejekce přítomna v 8% případů



BK 1%
ACR 5.2%
AMR 3.1%

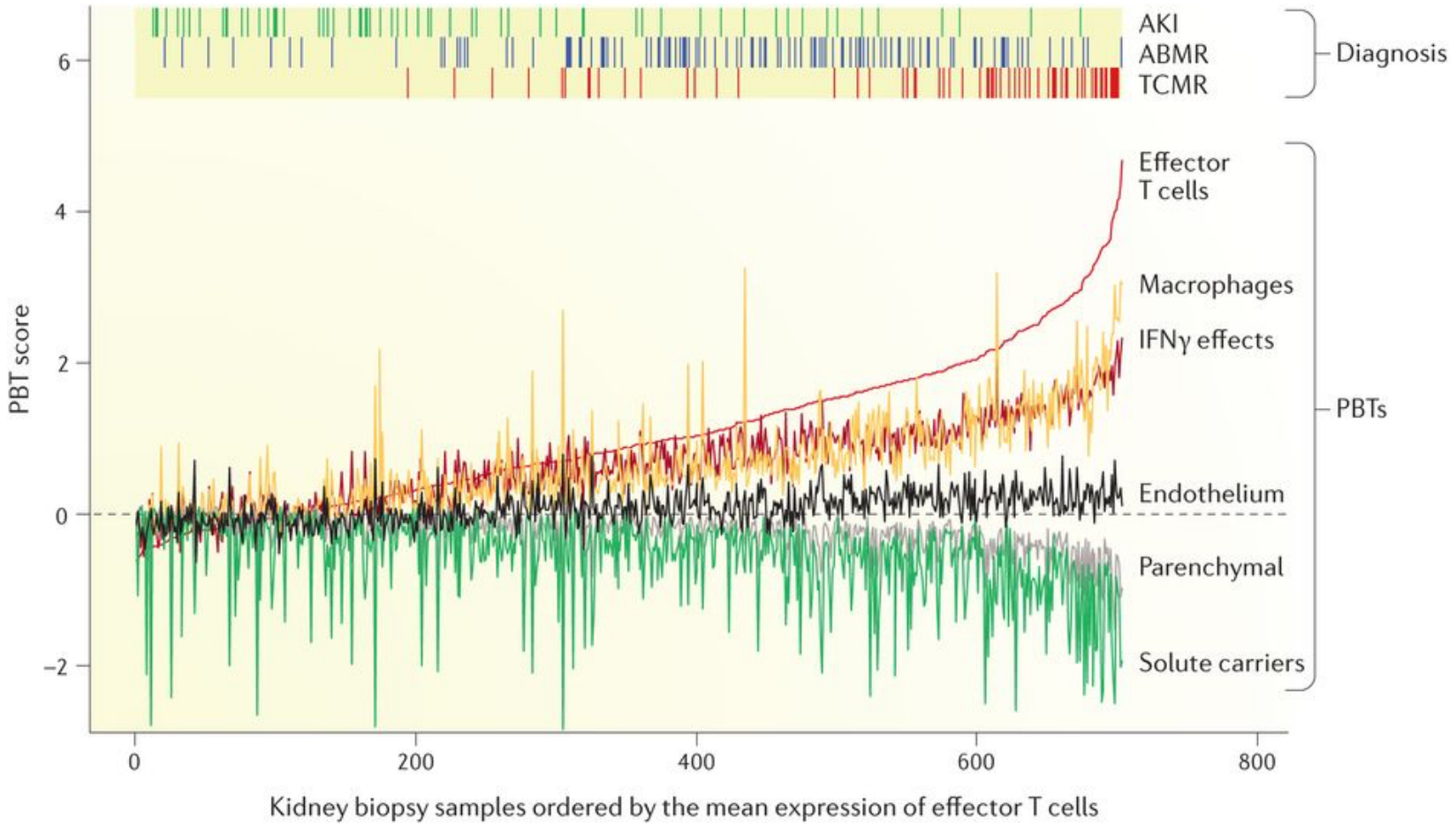
Izol. hraniční změny 18.6%

Possible sampling error in needle biopsies



Alexander C. Wiseman, James E. Cooper, https://abdominalkey.com/prophylaxis-and-treatment-of-kidney-transplant-rejection/

Molecular assesment allows diagnostics based on specific transcripts



Nature Reviews | Nephrology

Halloran P, et al. Nature Reviews Nephrology, 2016, 534–548

Explaining the Molecular Microscope® report for core kidney transplant biopsies (MMDx-Kidney)

Patient information
Date of transplant, date of biopsy, etc.

Clinical information
Time post-transplant; biopsy indication, DSA (if provided)

Additional detail
Rejection, injury-related binary classifiers and AKI transcript set

Comparison to normal
Scores of this biopsy interpreted vs. relatively normal biopsies

Clinical interpretation

Summary of molecular changes
(Injury, rejection)

Proportions rejection-related AA molecular changes
(Normal, TCMR, ABMR)

Visualization
Relationship of biopsy to others in reference set
PC2 vs. PC1

Visualization
PC2 vs. PC3

Survival of other kidneys like this one

% of biopsy that is cortex

Histologic and molecular diagnoses in the molecular nearest neighbors of this biopsy

Adherence index: Low scores in biopsies 6m-5y post-transplant correlate with possible non-adherence or under-immunosuppression

MMDx

Molecular Mi... ...e® Diagnostic Report for Kidney (MMDx-Kidney)

General information:

Surname	Unguran
Date of Birth	
Date Received (Y-M-D)	
Date Reported (Y-M-D)	
Date of Transplant (Y-M-D)	
Date of Biopsy (Y-M-D)	

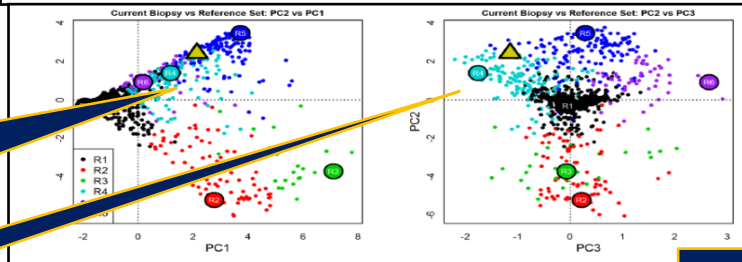
(Redacted)

Pure molecular interpretation

Abnormal biopsy. Severe early-stage ABMR with g and ptc-related molecular features. No TCMR. Mild inflammation, AKI and atrophy-fibrosis. Note that MMDx cannot exclude primary renal diseases.

	Classifier/gene sets[1,2]	Biopsy	Range of values[3]	Upper limit of normal[3]	Interpretation
Injury Scores	Inflammation Score[3]	-0.32	-3.8 – 5.8	0.03	Mild
	Acute Kidney Injury (AKI) Score[4]	0.16	-0.6 – 1.6	0.39	Mild
	Atrophy-Fibrosis Score[5]	0.33	0.0 – 1.0	0.82	Mild
Rejection Scores	Rejection Score[6]	0.74	0.0 – 1.0	0.30	Severe
	T Cell-Mediated Rejection (TCMR) Score[7,C]	0.01	0.0 – 1.0	0.10	Normal
	Antibody-Mediated Rejection (ABMR) Score,[8,C]	0.81	0.0 – 1.0	0.20	Severe

Rejection phenotype[3,D] (six scores, R1-R6, adding up to 1.0)				
	R1 Non-rejecting	0.00	All ABMR (Sum of R4, R5, and R6)	1.00
	R2 TCMR	0.00	*R4 Early-Stage ABMR (EABMR)*	0.59
	R3 Mixed Rejection	0.00	*R5 Fully-Developed ABMR (FABMR)*	0.41
			R6 Late-Stage ABMR (LABMR)	0.00



Survival in patients with similar biopsies in the Reference Set		Percent cortex[B,E]
1-year: 92%	3-years: 76%	96%

Clinical Notes

Molecul... ...pe® Diagnostic Report for Kidney (MMDx-...

	Classifier/G...	Biopsy score	Range of possible values[4]	Upper limit of normal[5]	Interpretation
TCMR related	TCMR-1[7]	0.01	0.0 – 1.0	0.10	Normal
	TCMR-2	0.01	0.0 – 1.0	0.10	Normal
	Mean of 2 TCMR classifiers	0.01	0.0 – 1.0	0.10	Normal
Rejection related	Rejection[6]	0.74	0.0 – 1.0	0.30	Severe
Injury-scarring related	AKI score[4]	0.16	-0.6 – 1.6	0.39	Mild
	Atrophy-Fibrosis Score[5]	0.33	0.0 – 1.0	0.82	Mild
ABMR related	ABMR-1[8]	0.82	0.0 – 1.0	0.20	Severe
	ABMR-2	0.77	0.0 – 1.0	0.20	Severe
	ABMR-3	0.84	0.0 – 1.0	0.20	Severe
	Mean of 3 ABMR classifiers	0.81	0.0 – 1.0	0.20	Severe
Classifiers based on histologic lesions	Glomerulitis (g) > 0 probability[3]	0.75	0.0 – 1.0	0.25	Severe
	Transplant glomerulopathy (cg) > 0 probability[3]	0.33	0.0 – 1.0	0.22	Mild
	Peritubular capillaritis (ptc) > 0 probability[3]	0.75	0.0 – 1.0	0.24	Severe
	DSA-positive probability	0.64	0.0 – 1.0	0.42	Moderate
	Interstitial inflammation (i) > 1 probability[3]	0.02	0.0 – 1.0	0.06	Normal
	Tubulitis (t) > 1 probability[3]	0.03	0.0 – 1.0	0.1	Normal
	Tubular atrophy (ct) > 1 probability	0.21	0.0 – 1.0	0.84	Normal
	Adherence index[11]	0.45	0.0 – 1.0	0.9	Normal

For classifiers: TCMR-1 = TCMR vs everything else; TCMR-2= TCMR vs everything else, with BK/Borderline/Mixed withheld; ABMR-1 = ABMR vs everything else with TG/ABMR suspicious withheld; ABMR-2 = ABMR and Mixed vs everything else, with TG/ABMR suspicious withheld; ABMR-3 = ABMR vs everything else, with Mixed/TG/ABMR suspicious withheld.

Rank order of the most common histologic diagnoses in the 50 nea... molecular neighbors	Mean molecular scores in the 50 nearest molecular neighbors
ABMR: 54% No Major Abnormalities (NOMOA): 12% Transplant Glomerulopathy (TG): 8% Mixed Rejection: 6% ABMR Suspicious: 6%	Rejection: 0.83 ABMR: 0.83 Atrophy-Fibrosis Score (cigt1): 0.28 AKI Score (IRRATs): 0.20 TCMR: 0.02

References for the scores, classifiers, and archetypes

1. Halloran PF et al. Nat... Reviews Nephrology 2016;12(9):534-...
2. Halloran PF et al. Kid... t 2014;(85):258-64.
3. Mueller TF et al. Am ... plant 2007;7(12):2712-22.
4. Famulski K et al. JASN... May;23(5):948-58.
5. Venner J et al. Journ... al Investigation Insight 2016;1(1)... 2.1172/jci.insight.85323.
6. Reeve J et al. Am J Tr... 009 Aug;9(8):1802-10.
7. Reeve J et al. Am J Tr... 13;13(3):645-55.
8. Sellares J et al. Am J ... 13;13(4):971-83.
9. Reeve J et al. JCI Insi... /insight.jci.org/articles/view...
10. Madill-Thomsen K et ... ant 2017 Feb 22;17(8):2117...

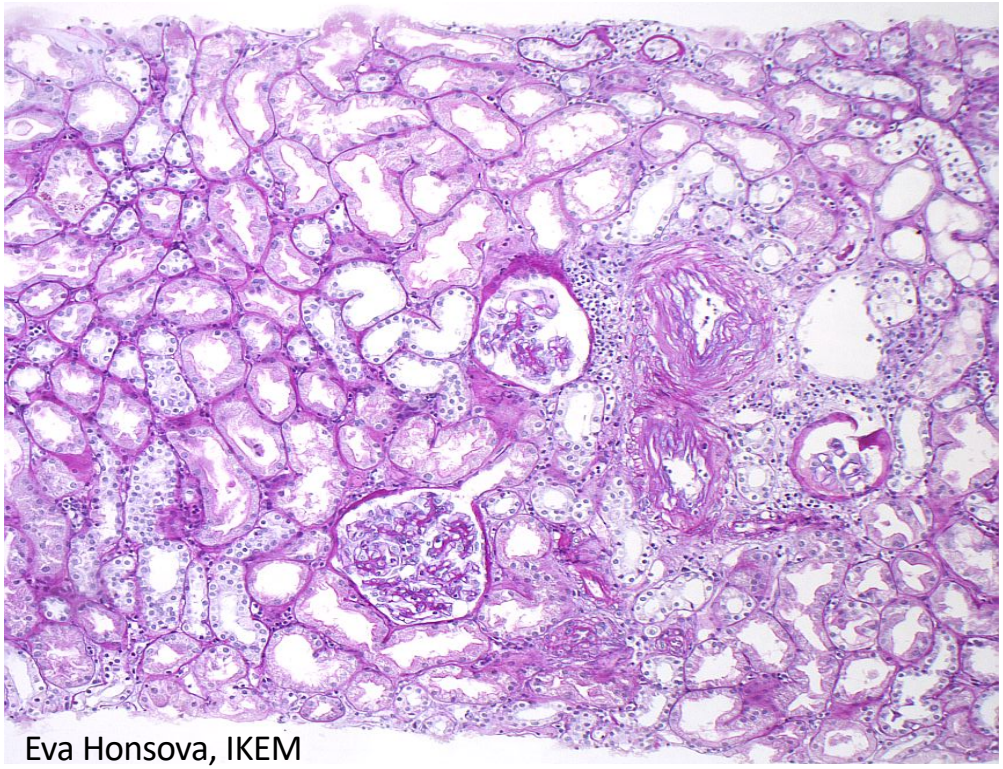
Historical case – MMDx from biobank

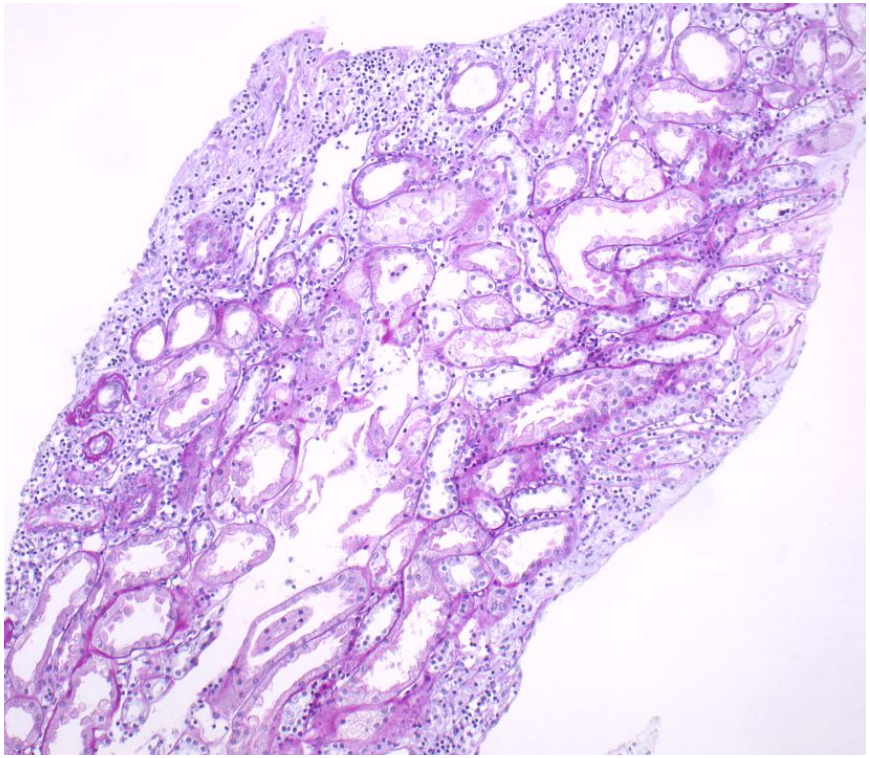
- 41 years, female
- 1st deceased donor kidney transplantation
- ECD, age 65
- DSA negative, peak PRA 4%, HLA mismatch 3
- no induction

7th POD: DGF, kidney graft biopsy:

T-cell mediated rejection, IIA with mild interstitial component (C4d negative)

Therapy: 2g of methylprednisolone, S-Cr 200µmol/l (2.26mg/dl)





Eva Honsova, IKEM

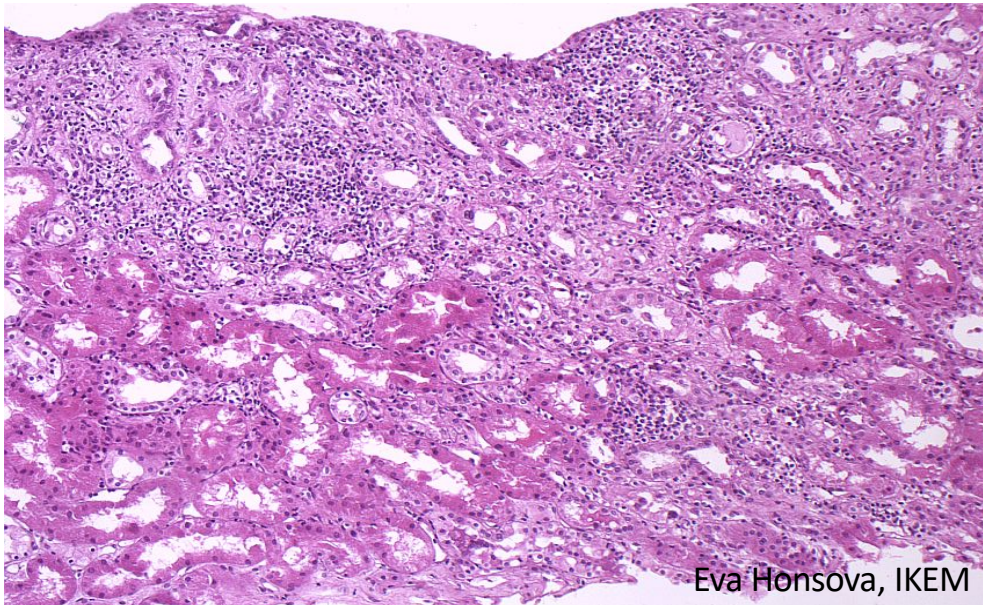
IKEM

3M protocol biopsy
S-Cr: 156umol/l (1.76mg/dl)
Interstitial inflammation with tubulitis,
formation of fibrosis
T-cell mediated rejection IA (C4d negative, polyoma negative)
g1 i2 ti2 t2 cv2 ah2

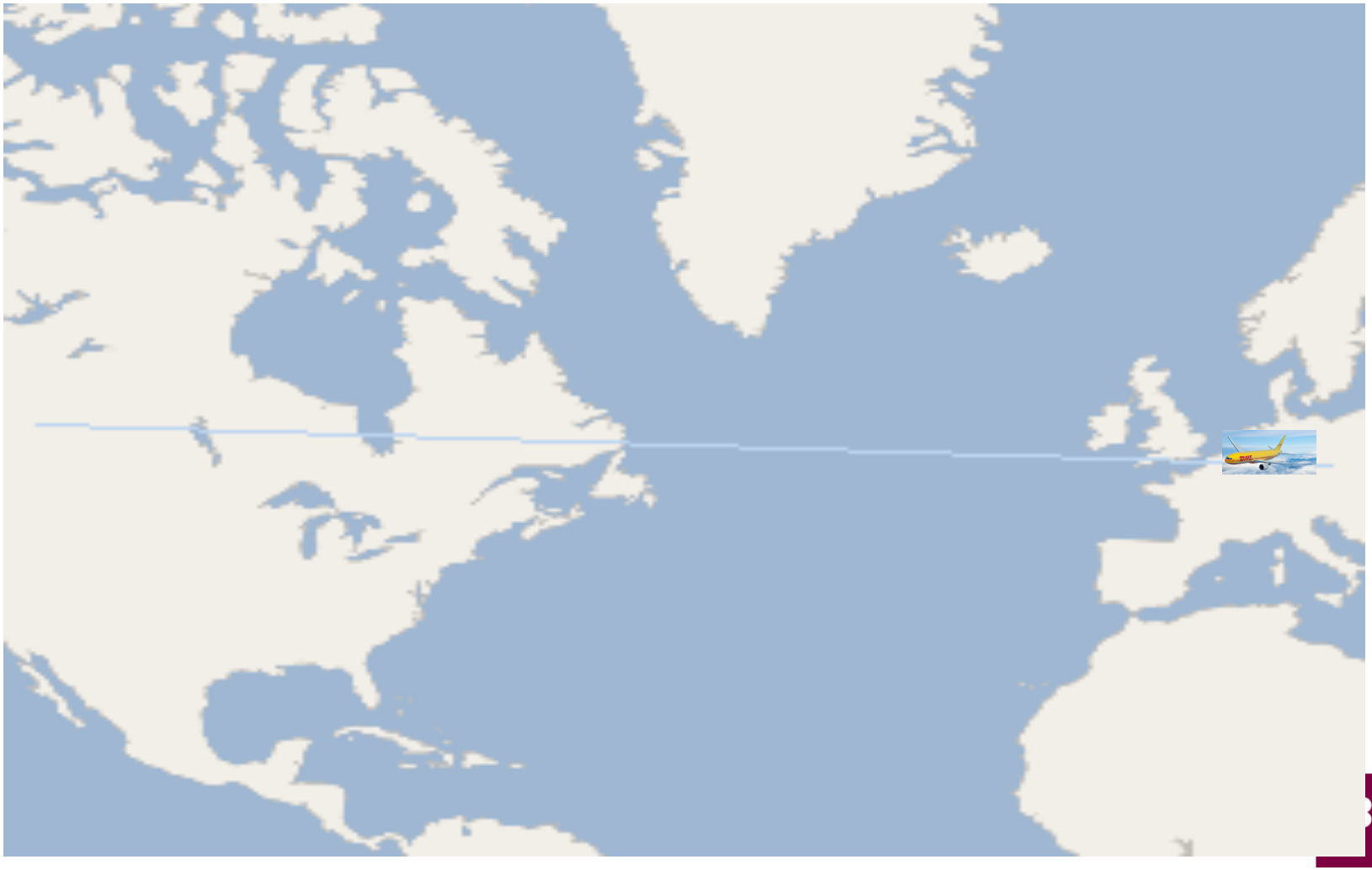
Thymoglobuline 325mg (cummulative dose of 3.7 mg/kg)

FU: 5.5 years after Tx
Stable S-Cr 178.5 µmol/l

MMDx from the biobank



MMDx in Edmonton

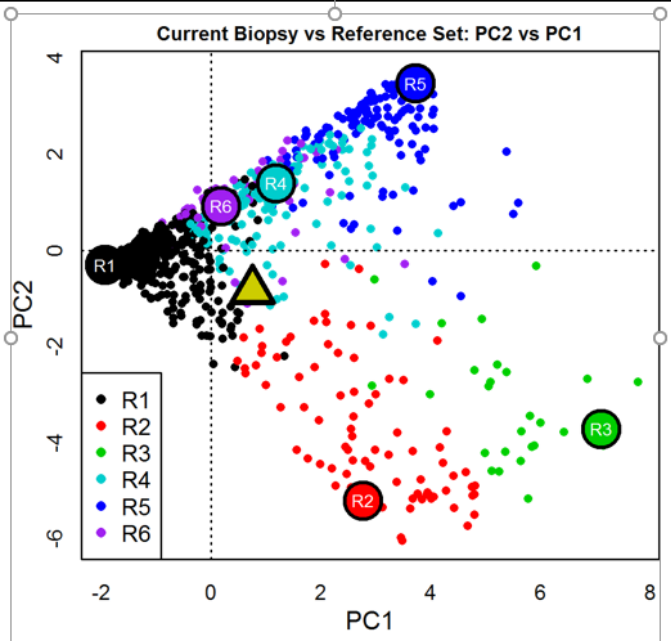


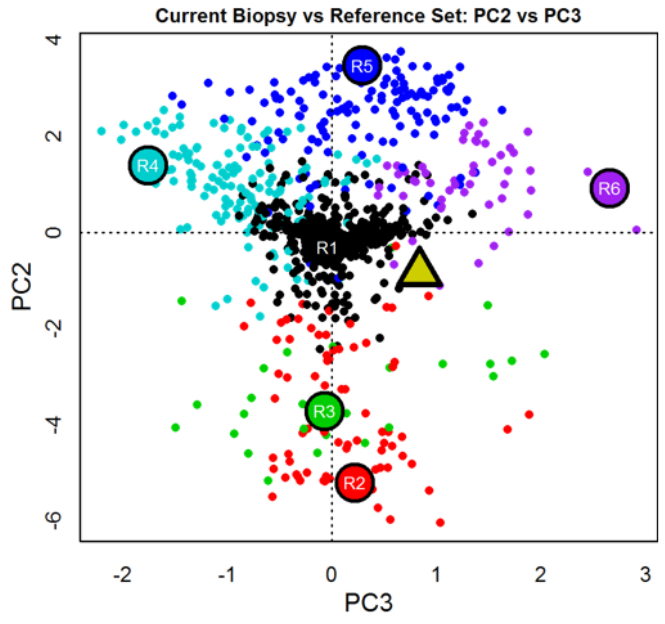
Banff histology
g1 i2 ti2 t2 cv2 ah2

MMDx from historical sample:
No rejection

	Classifier/gene sets[1, 2]	Biopsy	Range of values[A]	Upper limit of normal[B]	Interpretation
Injury Scores	Global Disturbance Score[3]	9.07	-3.8 – 5.8	0.03	Extensive
	Acute Kidney Injury (AKI) Score[4]	0.88	-0.6 – 1.6	0.69	Moderate
	Atrophy-Fibrosis Score[5]	0.16	0.0 – 1.0	0.39	Minimal
Rejection Scores	Rejection Score[6]	0.06	0.0 – 1.0	0.30	Normal
	T Cell-Mediated Rejection (TCMR) Score[7, C]	0.09	0.0 – 1.0	0.10	Normal
	Antibody-Mediated Rejection (ABMR) Score[8, C]	0.07	0.0 – 1.0	0.20	Normal

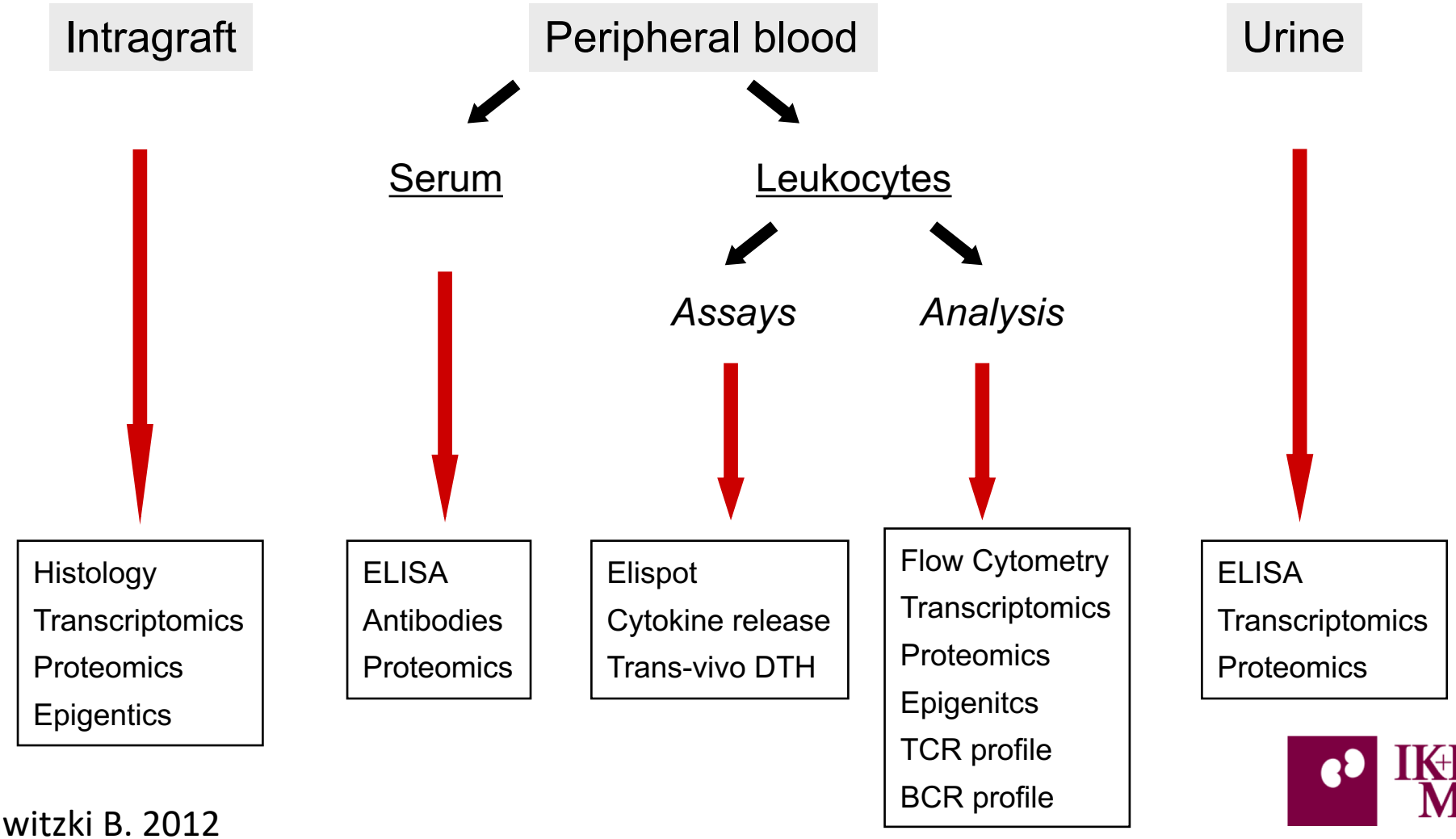
Rejection phenotype[9, D] (six scores, R1-R6, adding up to 1.0)	R1 Non-rejecting	0.14	All ABMR (Sum of R4, R5, and R6)	0.59
	R2 TCMR	0.27	*R4 Early-Stage ABMR (EABMR)*	0.19
	R3 Mixed Rejection	0.00	*R5 Fully-Developed ABMR (FABMR)*	0.00
			R6 Late-Stage ABMR (LABMR)	0.40





Možnosti predikce osudu štěpu

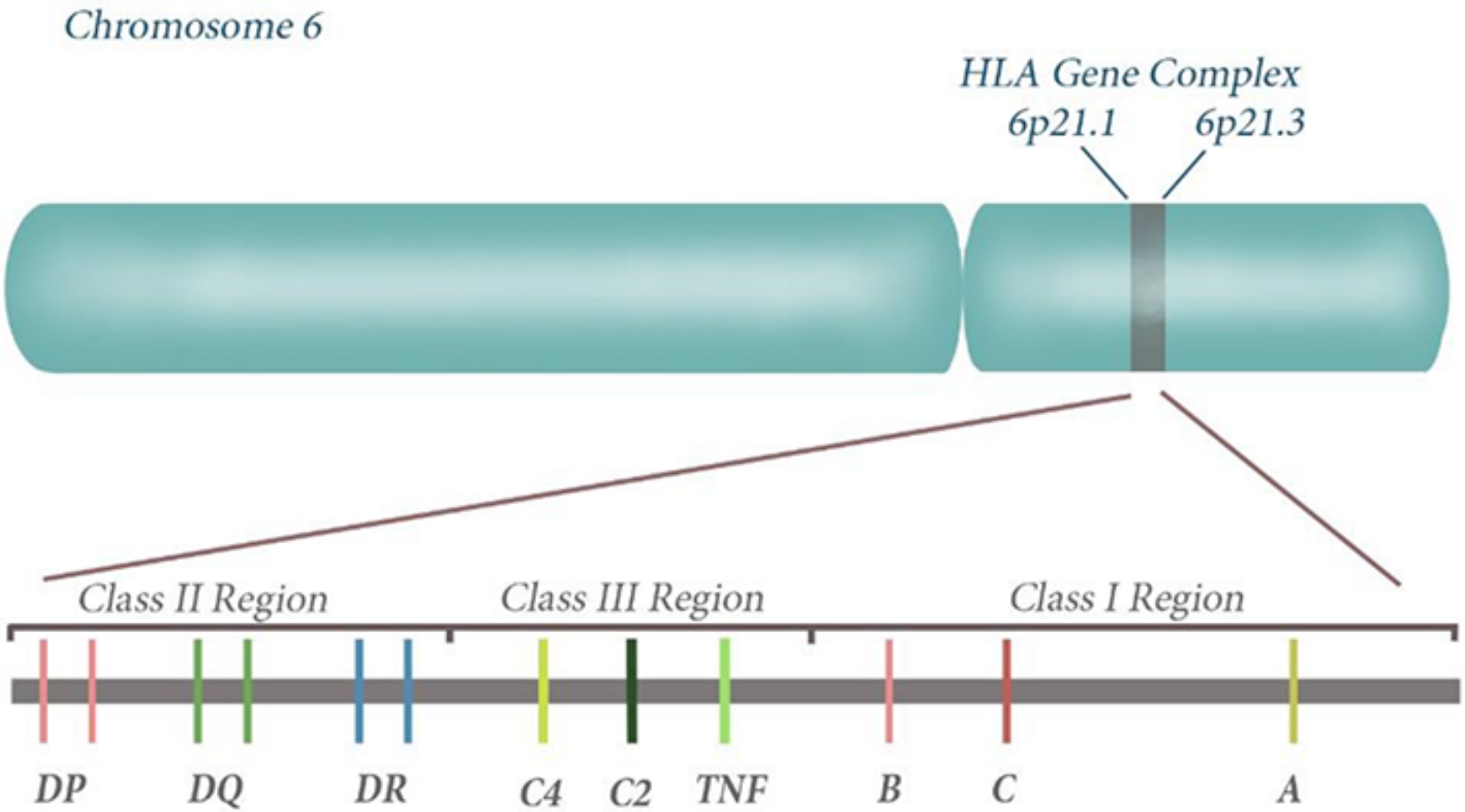
Potenciální eseje pro před/potransplantační monitorování



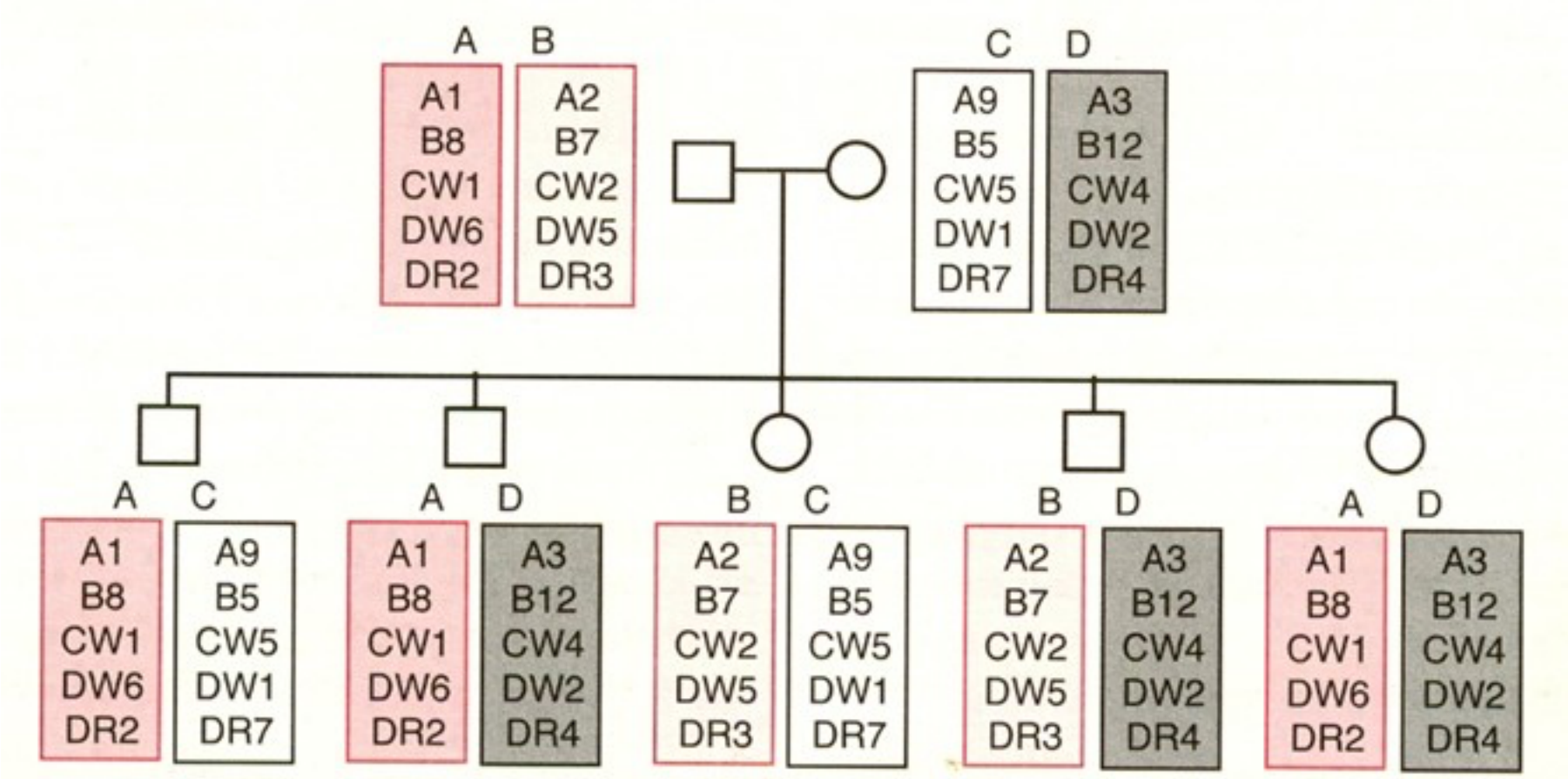
Sawitzki B. 2012

IKEM

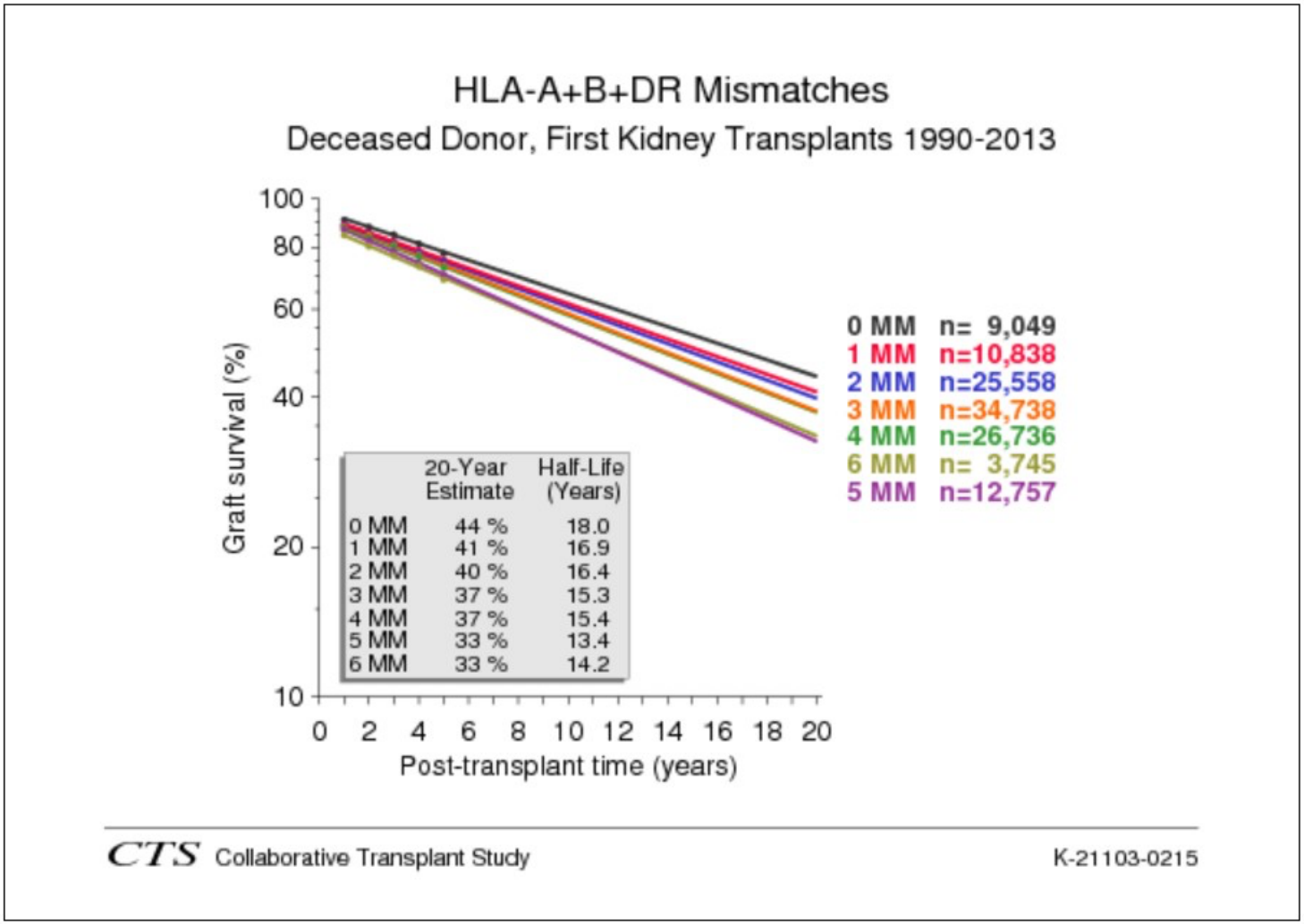
Lidský HLA systém je kódován na 6 chromozomu



Dědičnost HLA haplotypů

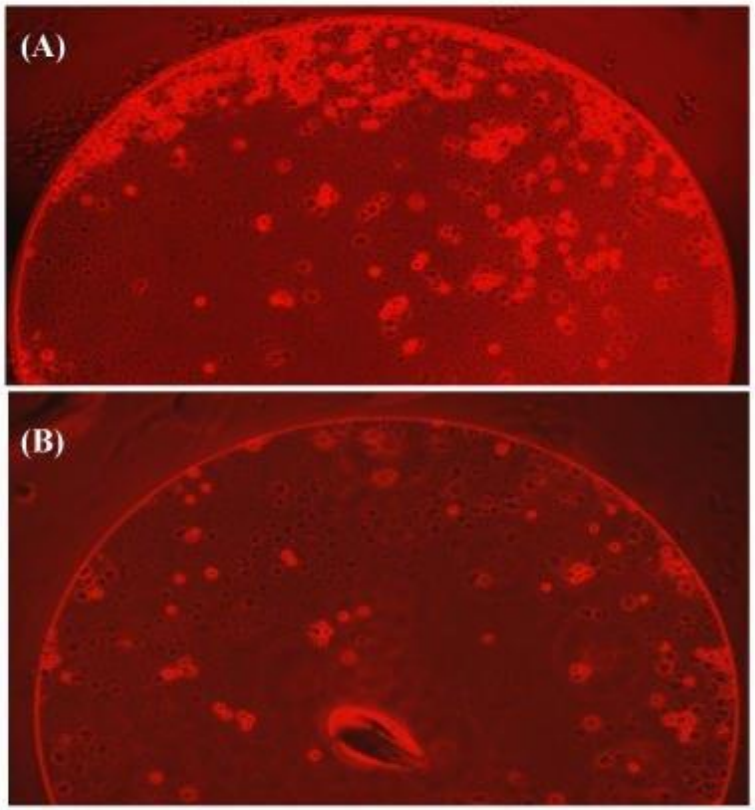


Více je lépe…

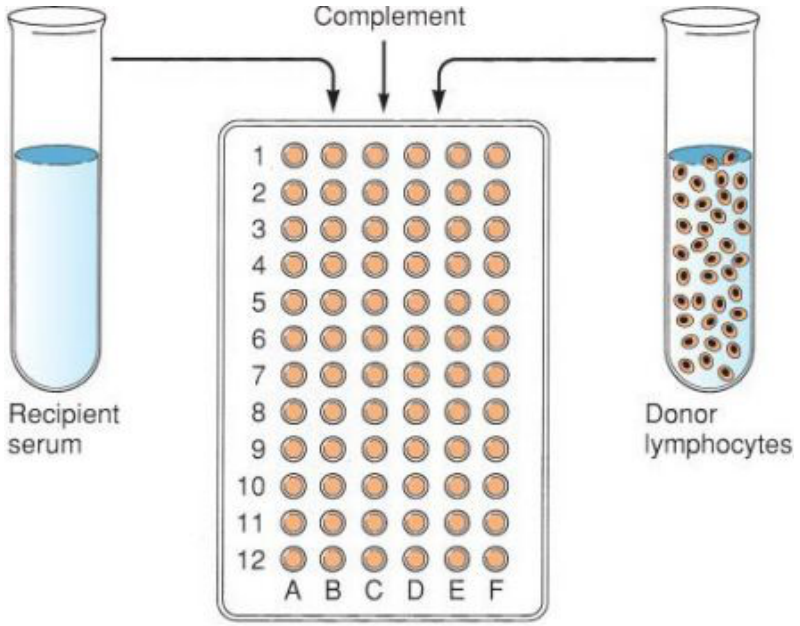

HLA-A+B+DR Mismatches
Deceased Donor, First Kidney Transplants 1990-2013
Graft survival (%)
100
80
60
40
20
10
0 2 4 6 8 10 12 14 16 18 20
Post-transplant time (years)
0 MM n= 9,049
1 MM n=10,838
2 MM n=25,558
3 MM n=34,738
4 MM n=26,736
6 MM n= 3,745
5 MM n=12,757
20-Year Estimate
Half-Life (Years)
0 MM 44 % 18.0
1 MM 41 % 16.9
2 MM 40 % 16.4
3 MM 37 % 15.3
4 MM 37 % 15.4
5 MM 33 % 13.4
6 MM 33 % 14.2
CTS Collaborative Transplant Study
K-21103-0215

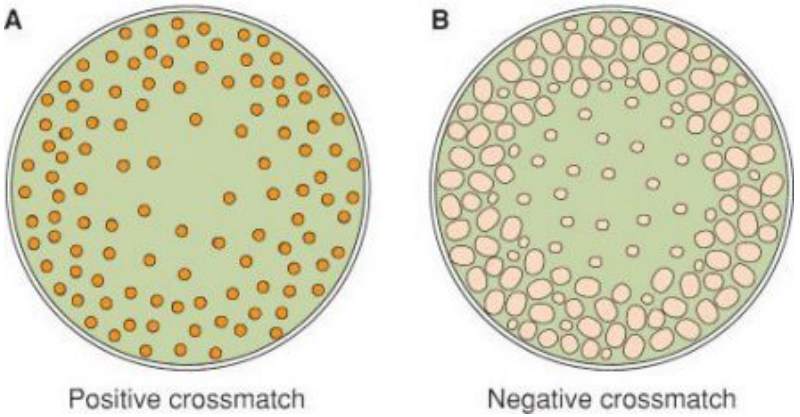
Complement dependent cytotoxicity test (cross-match)

- Since 1970s prospective lymphocyte cross-match is a routine in kidney transplantation for prevention of DSA damage



Panel reactive antibodies (PRA)

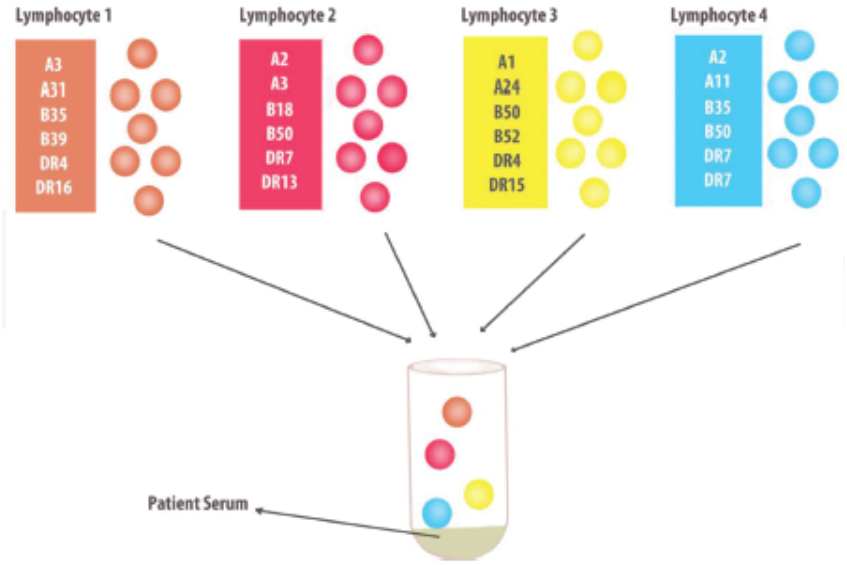




- Serum screening for preformed antibodies using a panel of typing cells
- Techniques identical to cross match
- Expressed as a percentage of cells with which the patient's serum reacts (0% to 100%)
- Sensitization (or increased panel reactive antibody) occurs as a consequence of prior pregnancy, blood transfusion, or transplant

IK+E M

Konstrukce panelu HLA antigenů



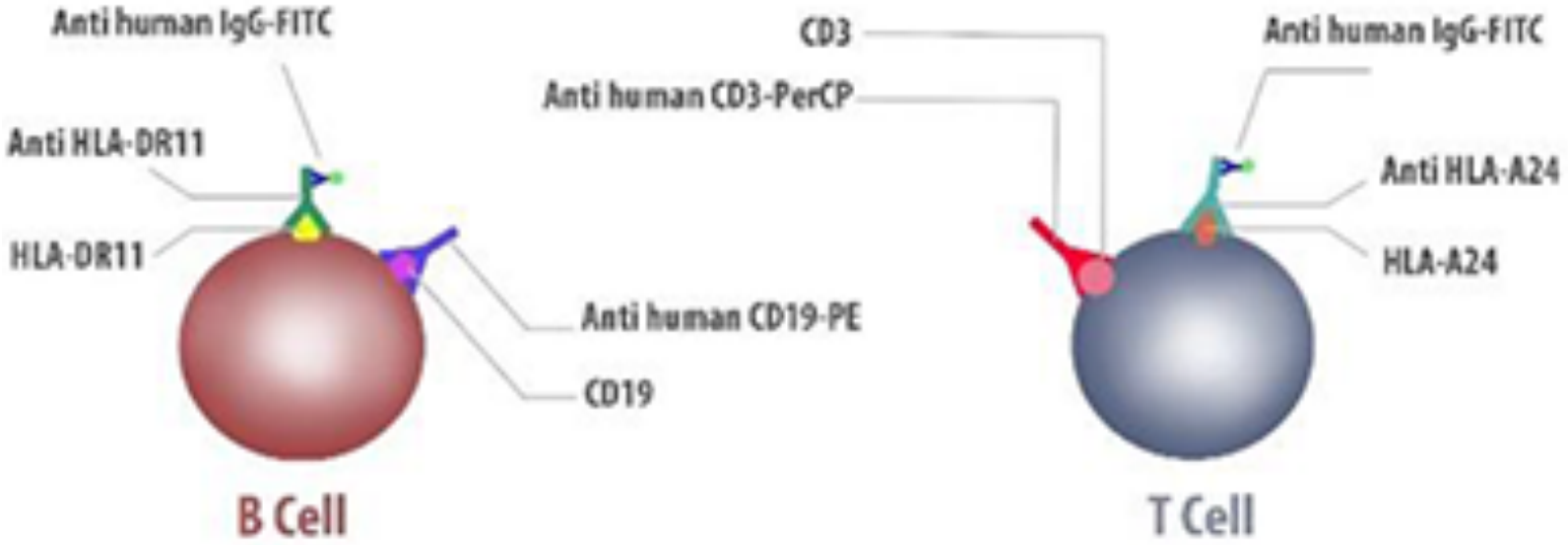
Specif icity	Number in panel	Speci ficity	Number in panel	Specif icity	Number in panel	Specif icity	Number in panel
A1	4	A80	2	B51	2	B75	1
A2	5	B7	2	B52	1	B78	1
A3	5	B8	2	B53	1	B81	2
A11	5	B13	2	B54	1	Bw4	18
A23	3	B18	2	B55	2	Bw6	26
A24	4	B27	2	B56	1	Cw1	6
A25	2	B35	2	B57	2	Cw2	6
A26	2	B37	2	B58	2	Cw4	4
A29	2	B38	1	B59	1	Cw5	1
A30	3	B39	1	B60	2	Cw6	7
A31	2	B41	2	B61	2	Cw7	9
A32	3	B42	1	B62	2	Cw8	5
A33	3	B44	2	B63	1	Cw9	2
A34	2	B45	1	B64	1	Cw10	4
A36	2	B46	1	B65	1	Cw12	1
A66	3	B47	2	B67	1	Cw14	2
A68	2	B48	2	B71	1	Cw15	3
A69	1	B49	1	B72	2	Cw16	2
A74	2	B50	1	B73	1	Cw17	3
PRA I screening Lot 017-One Lambda						Cw18	3

Techniques for detection of HLA antibodies

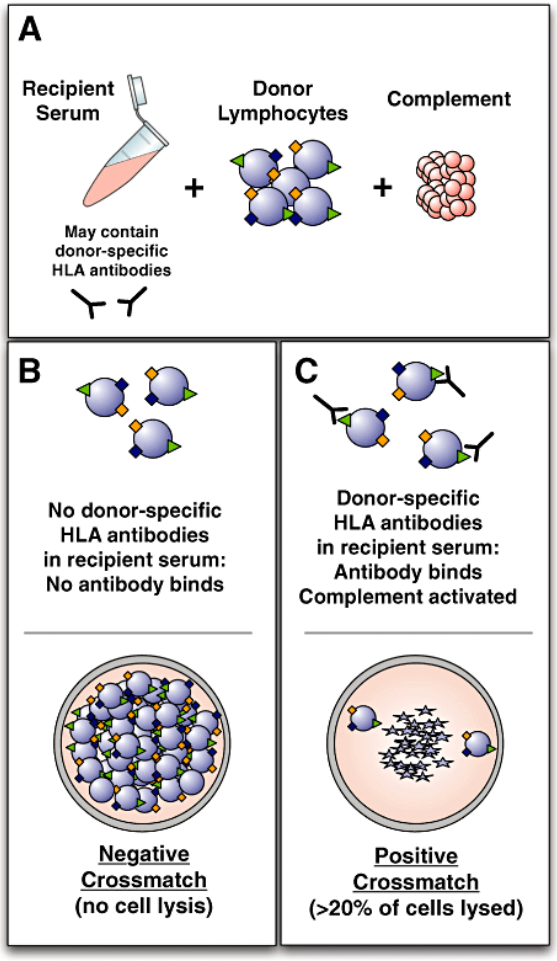
Technology	Testing	Advantages	Limitations	Sensitivity	Specificity
Complement-dependent cytotoxic crossmatch	Donor lymphocytes are incubated with candidate serum and complement added	Highly predictive for hyperacute rejection	Subjective Cannot detect noncomplement binding or low-level antibodies	+	+++
Flow cytometric crossmatch	Donor T and B lymphocytes are exposed to candidate serum	Semiquantitative. Increased sensitivity to low-level antibody	Higher sensitivity may lead to false-positive results	++	++
Solid-phase assays: bead based	Purified HLA molecules immobilized onto solid surface	Can detect specific antigens a candidate has antibodies against	Lack of standardization Significant interlaboratory variability. Unclear significant result	++++	+

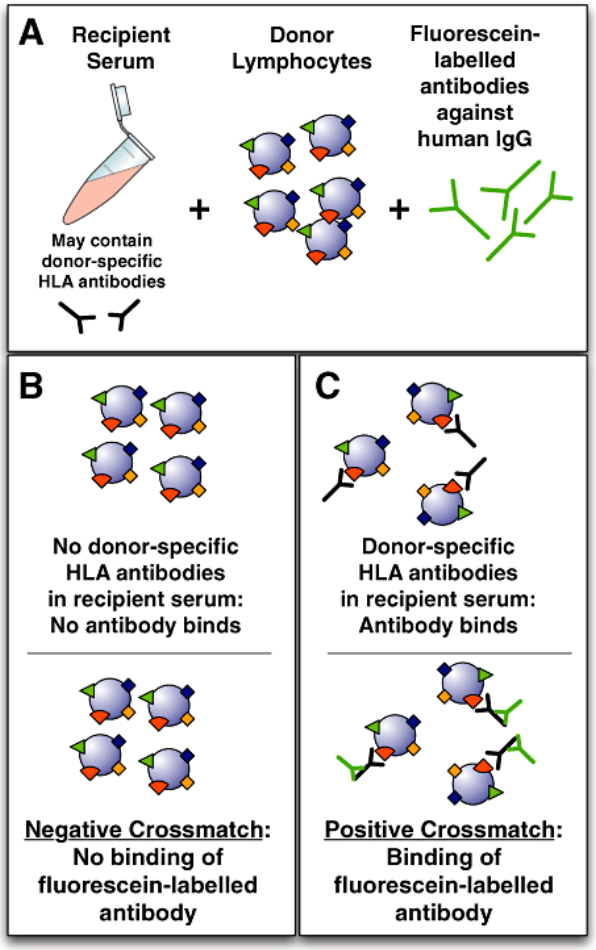
Keith, Vranic. Clin J Am Soc Nephrol. 2016 Apr 7; 11(4): 684–693

Princip metody FCXM (flow cytometric crossmatch)



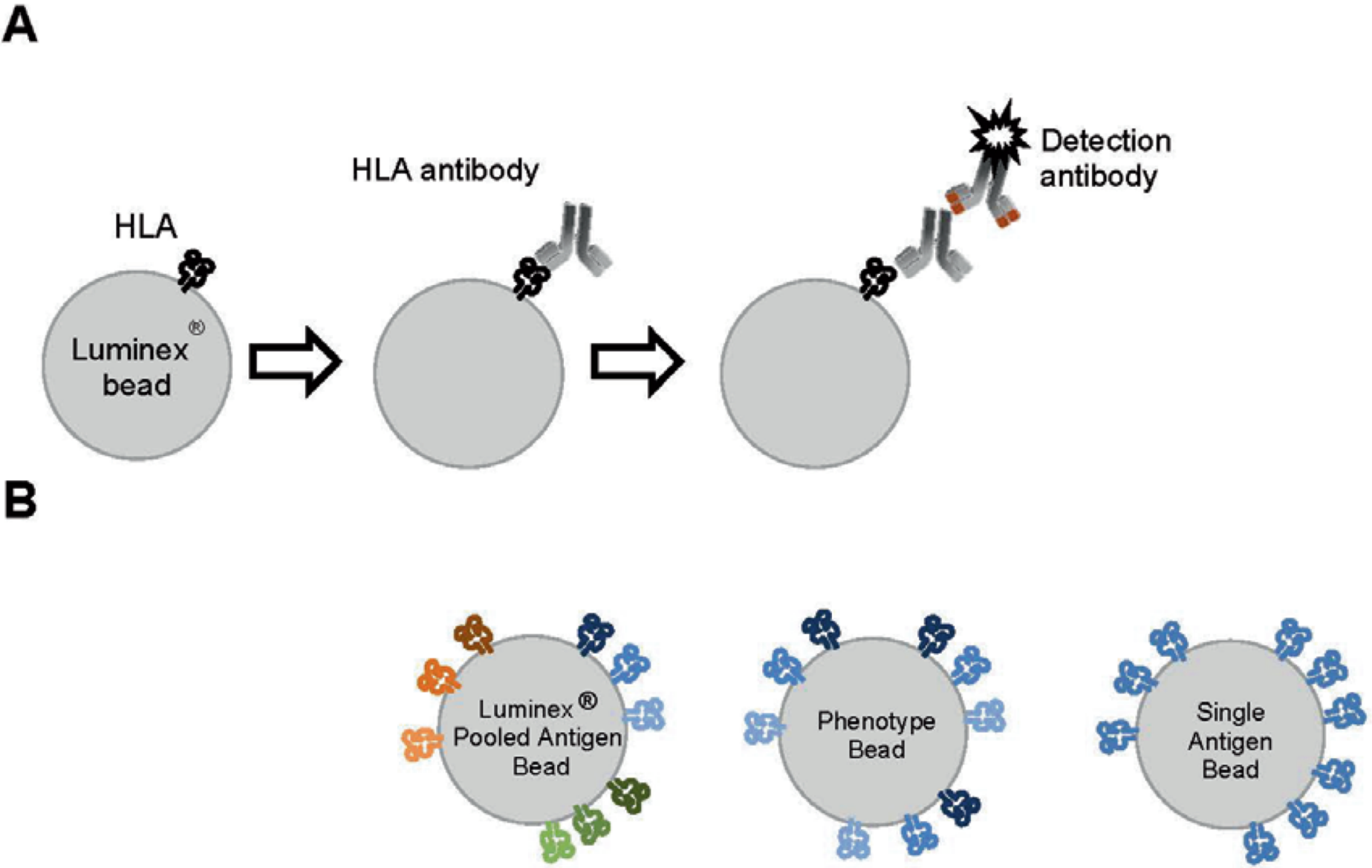
Crossmatch testing in organ transplantation: CDC crossmatch and flow-cytometry crossmatch

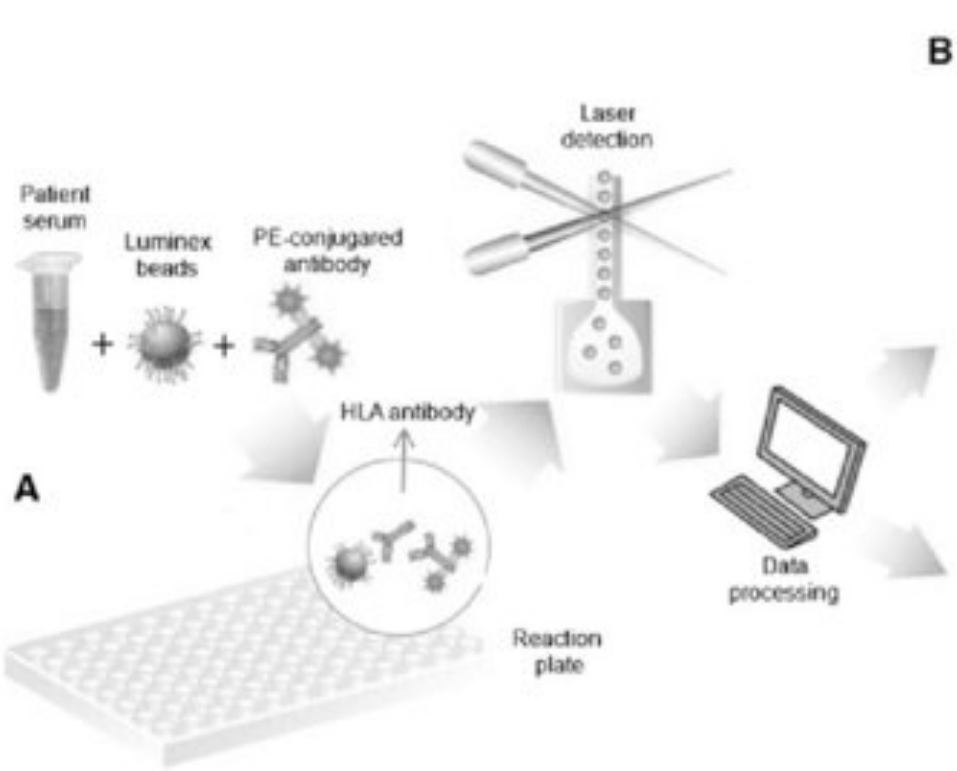


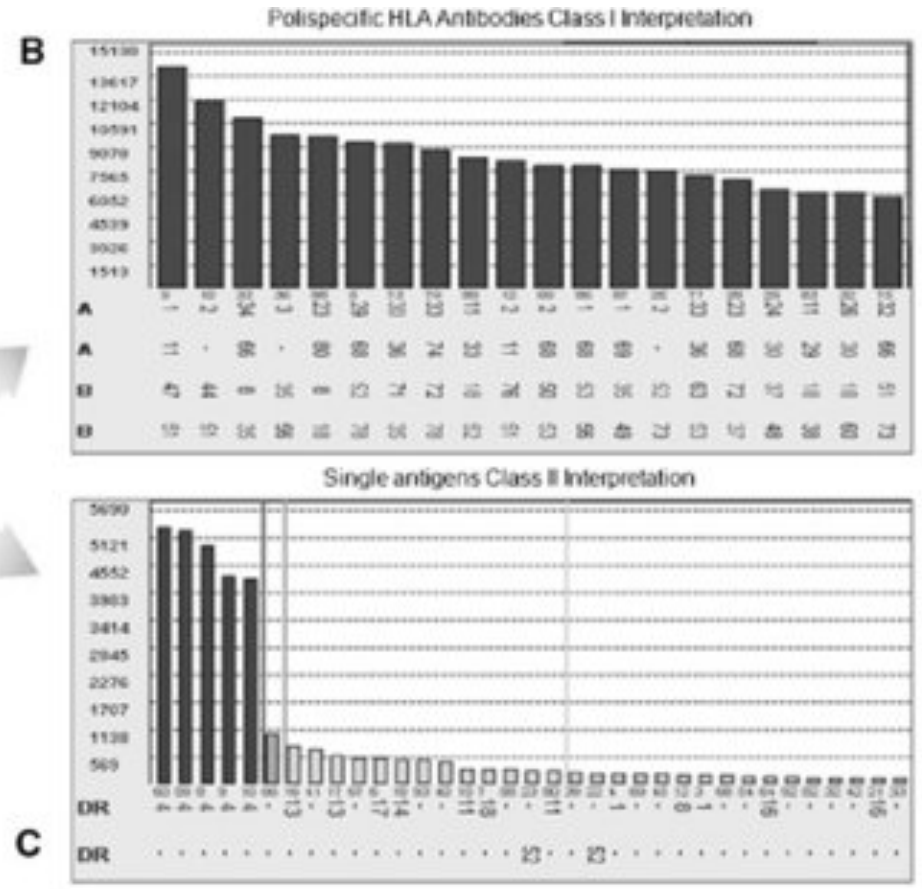


Nephrology
Volume 16, Issue 2, pages 125-133, 27 JAN 2011 DOI: 10.1111/j.1440-1797.2010.01414.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1440-1797.2010.01414.x/full#f1

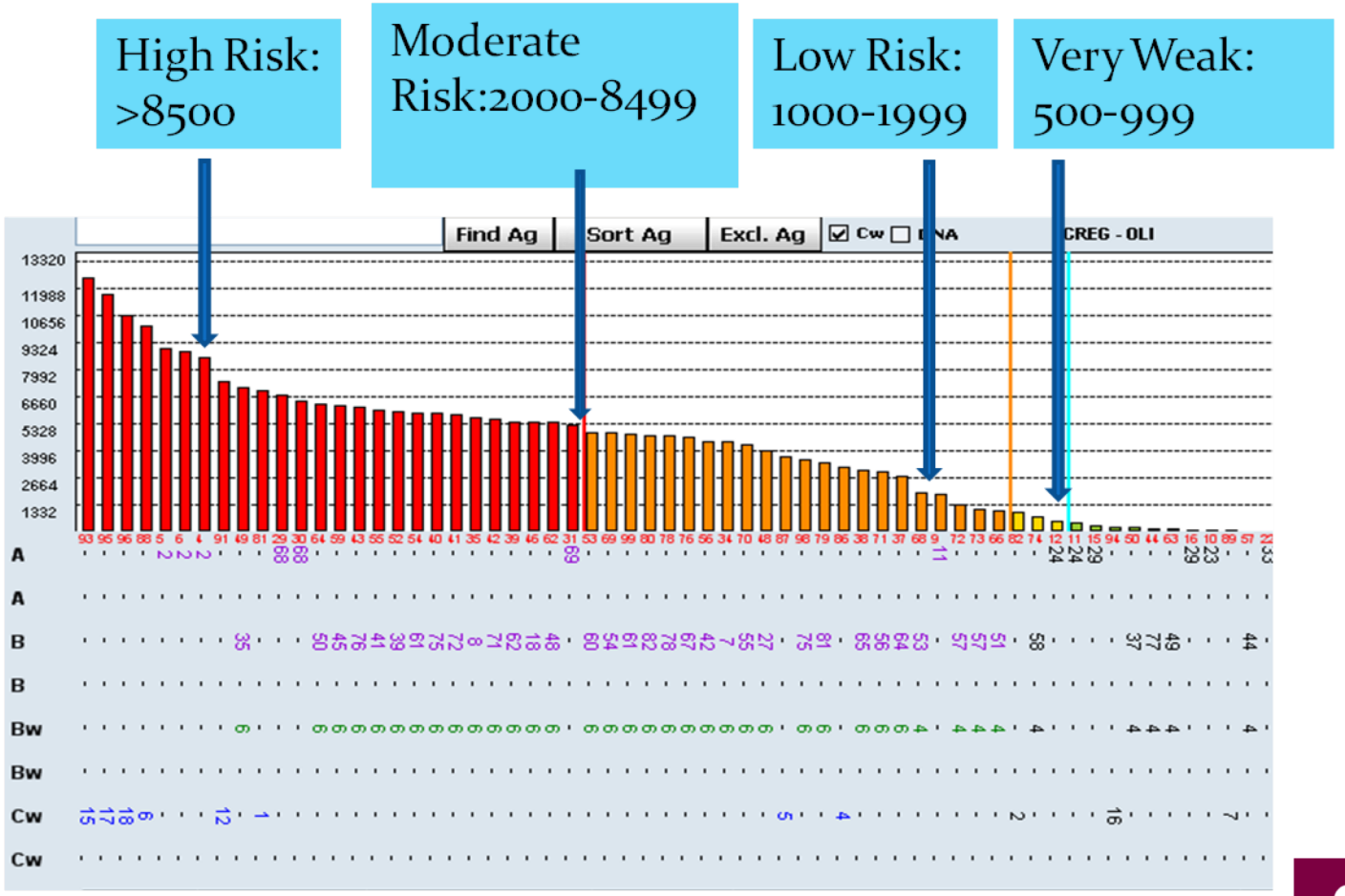
Princip metody Luminex



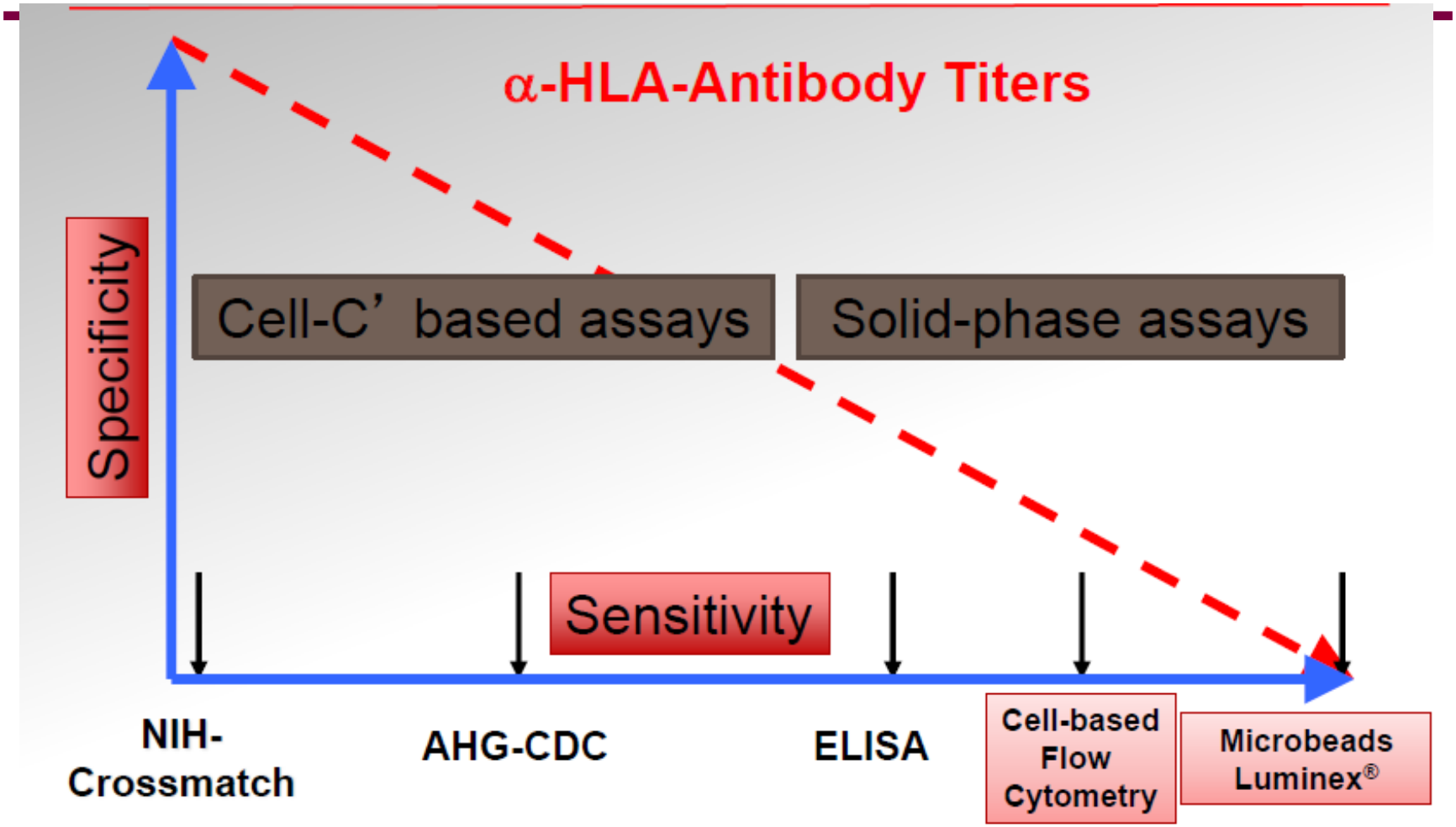

Patient serum
Luminex beads
PE-conjugated antibody
Laser detection
HLA antibody
A
Data processing
Reaction plate


B
Polispecific HLA Antibodies Class I Interpretation
C
Single antigens Class II Interpretation

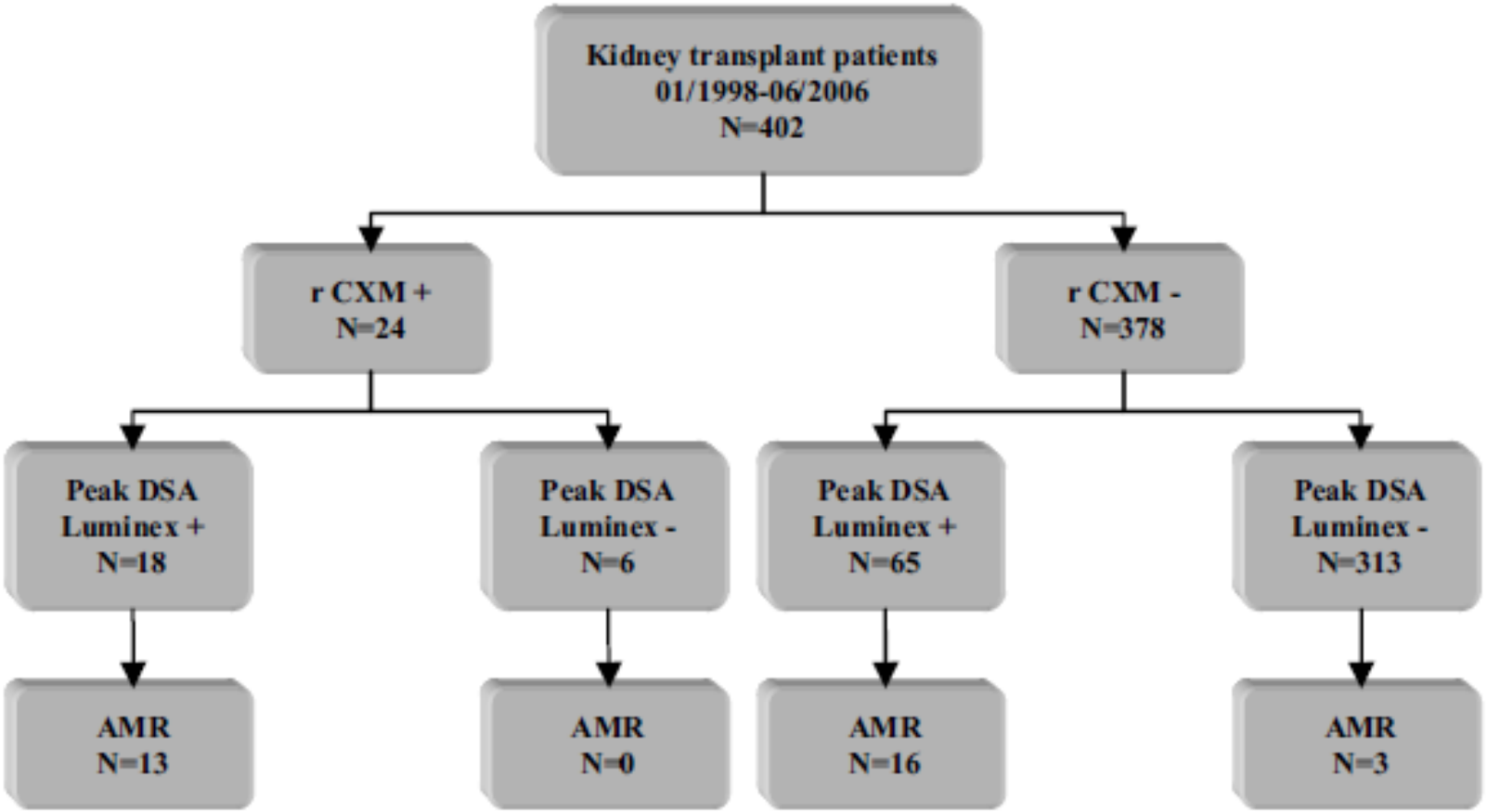
Luminex single antigen beads



Sensitivita testů k detekci HLA protilátek

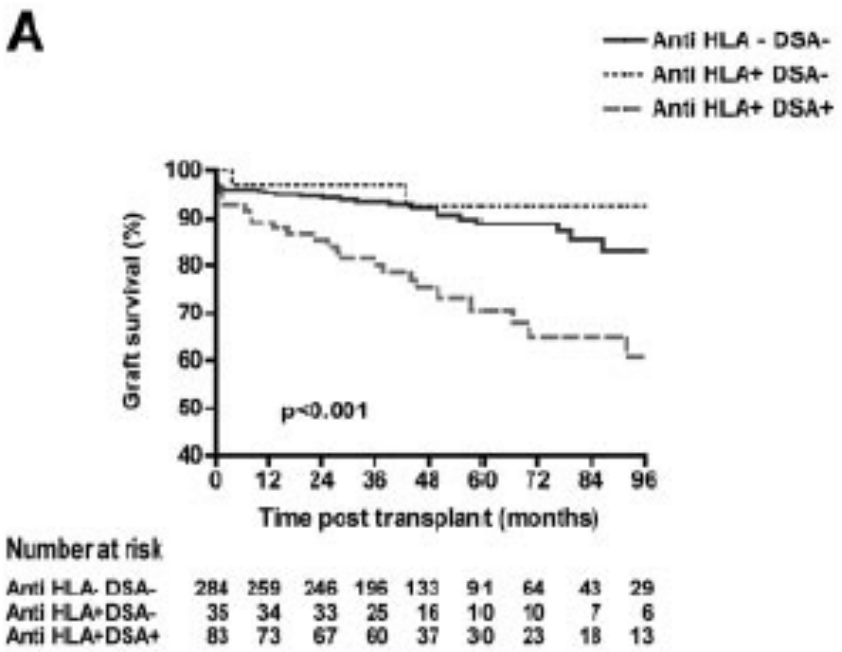


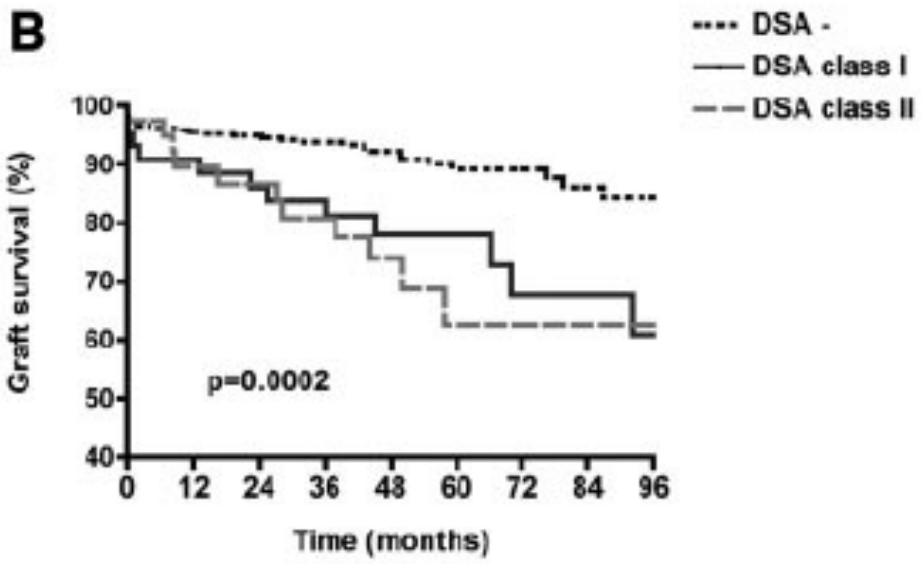
Mnoho CDC negativních nemocných má DSA před transplantací



Lefaucheur C. et al. J Am Soc Nephrol. 2010 ;21:1398-406.

Přítomnost DSA před transplantací koreluje s výsledky

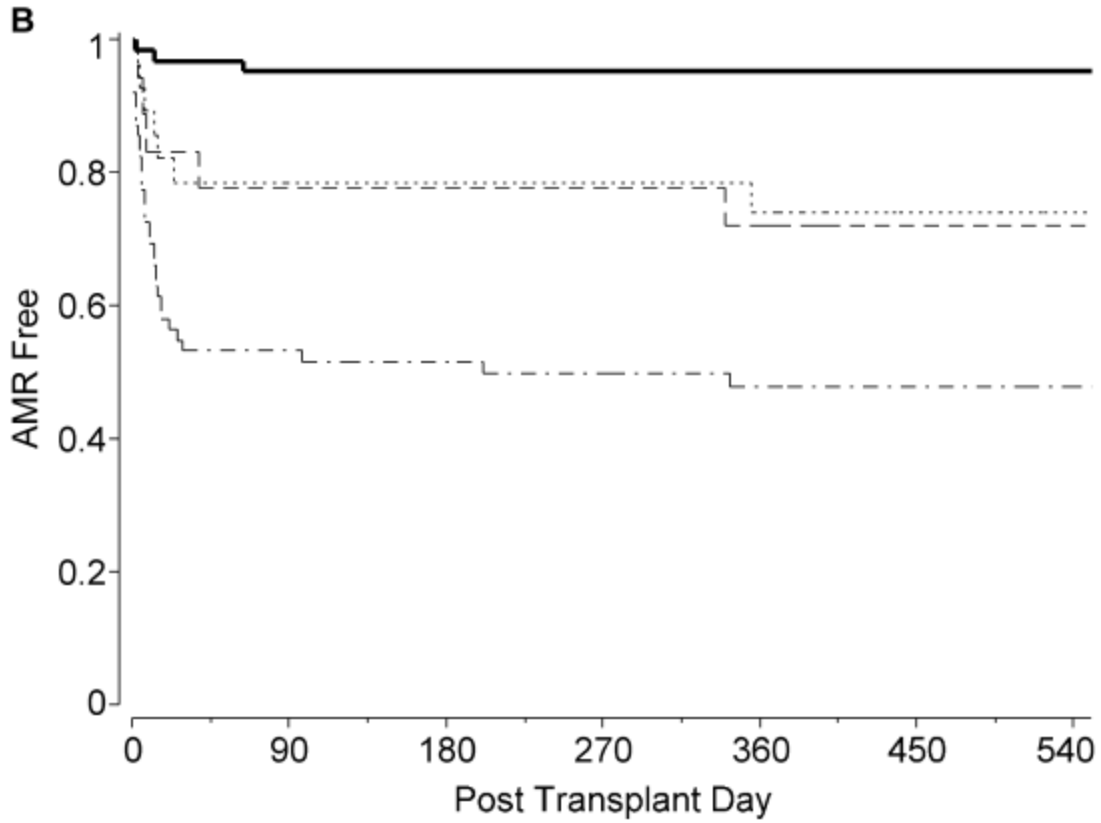




Lefaucheur C. et al. J Am Soc Nephrol. 2010 ;21:1398-406.

Baseline Donor-Specific Antibody Levels and Outcomes in Positive Crossmatch Kidney Transplantation

J. M. Gloor[a,*], J. L. Winters[b], L. D. Cornell[b], L. A. Fix[c], S. R. DeGoey[b], R. M. Knauer[b], F. G. Cosio[a], M. J. Gandhi[b], W. Kremers[d] and M. D. Stegall[c]



No DSA identified

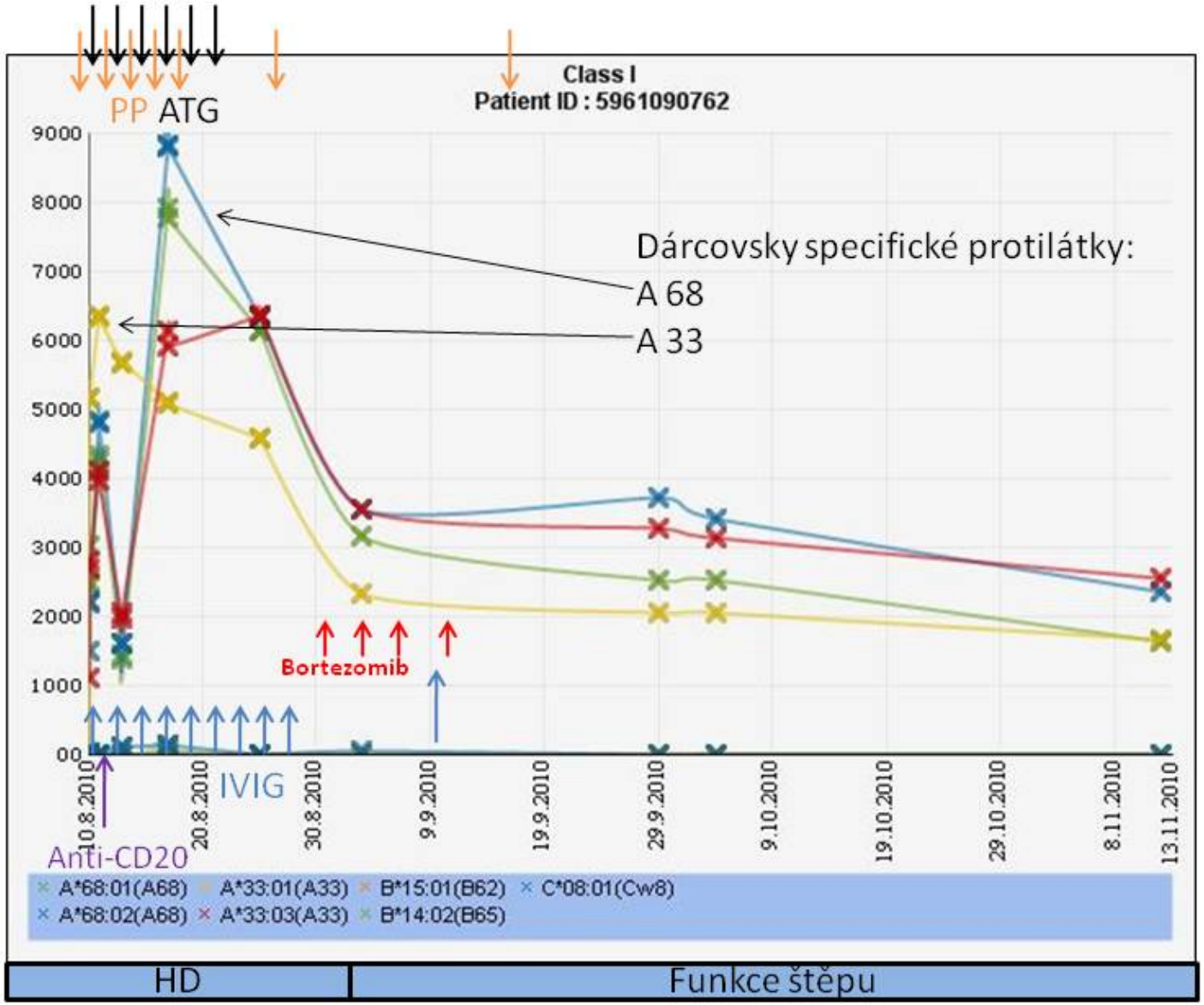
MFI <5,000 and >0
MFI >5,000 and <10,000

MFI >10,000

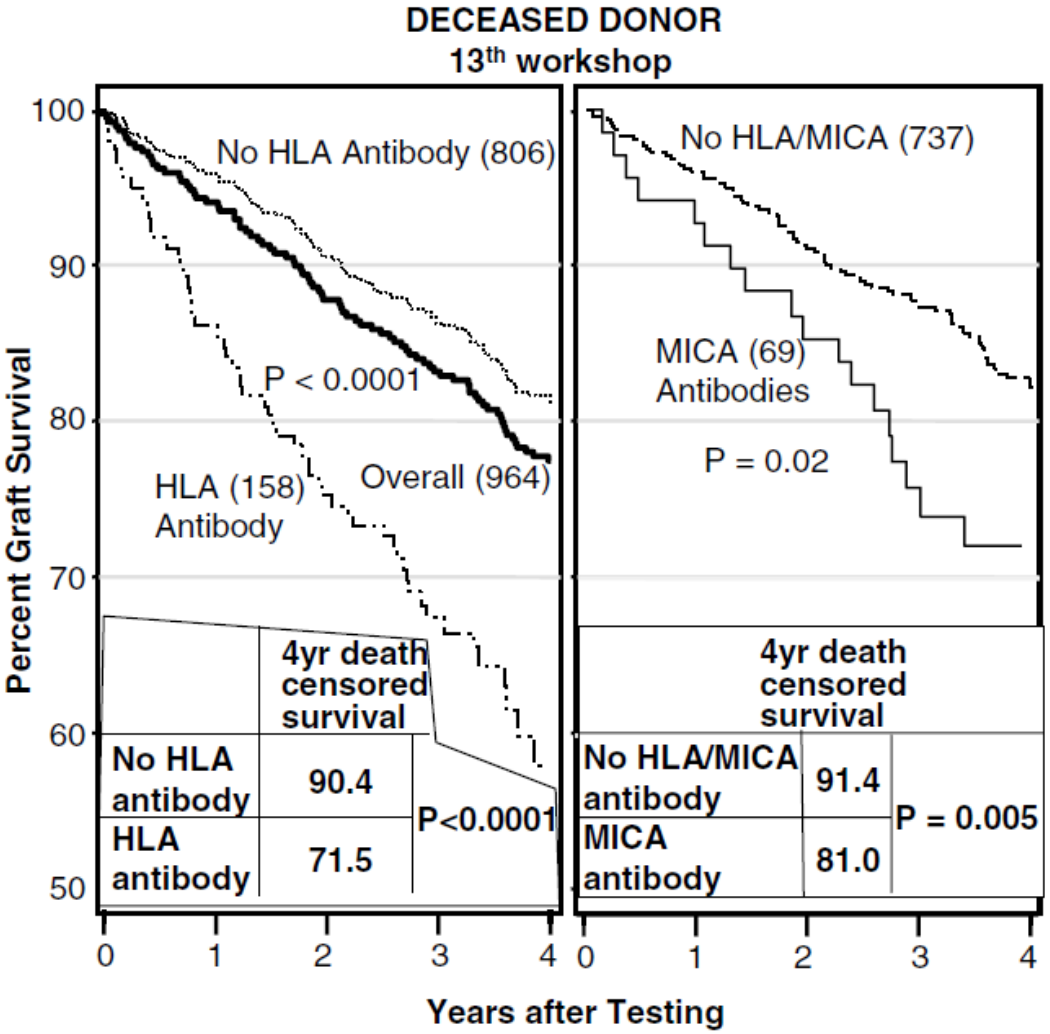
American Journal of Transplantation 2010; 10: 582–589

Kidney transplantation in the presence of donor specific antibody (DSA)

IKEM 2010

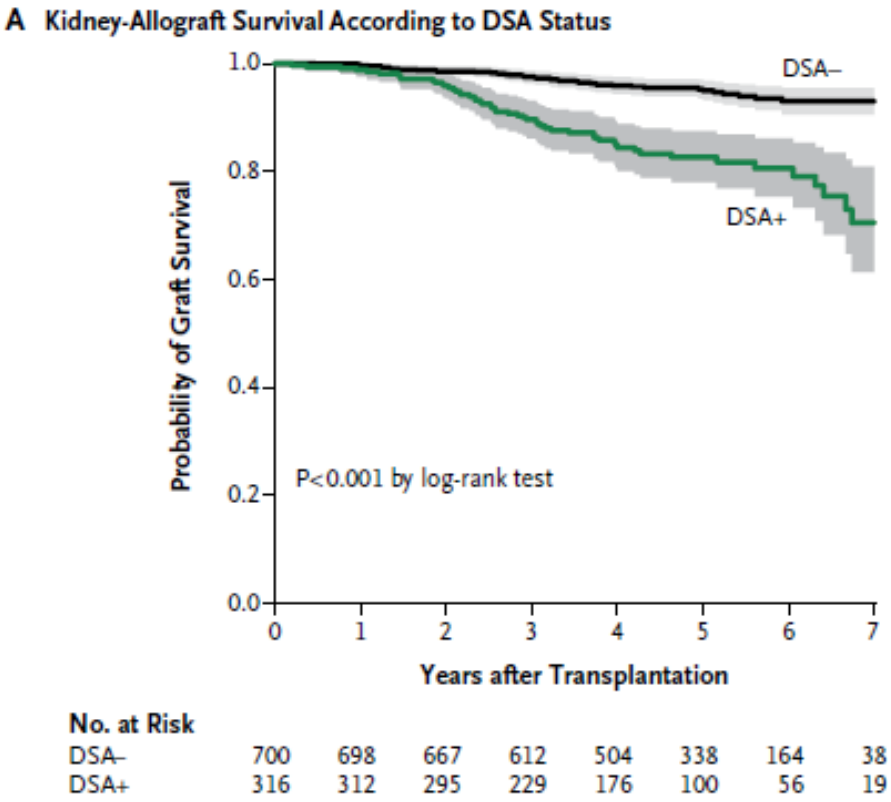


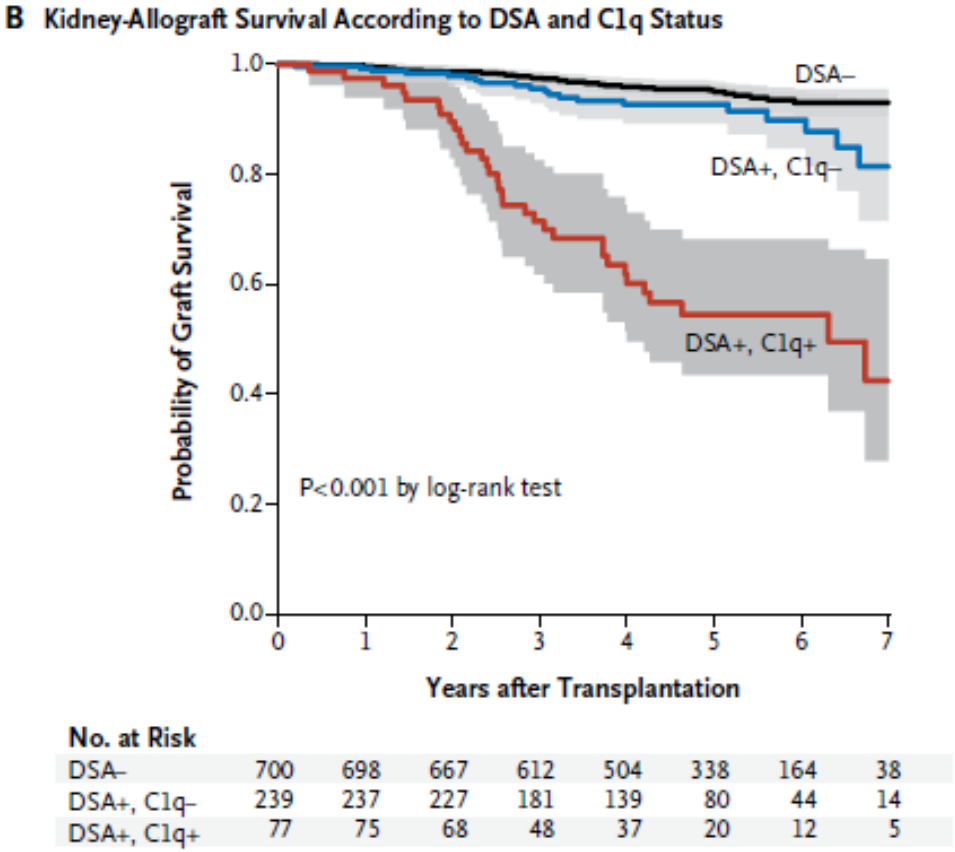
HLA and MICA antibodies and kidney graft survival



Terasaki PI et al. Am J Transplant 2007;7:408-415.

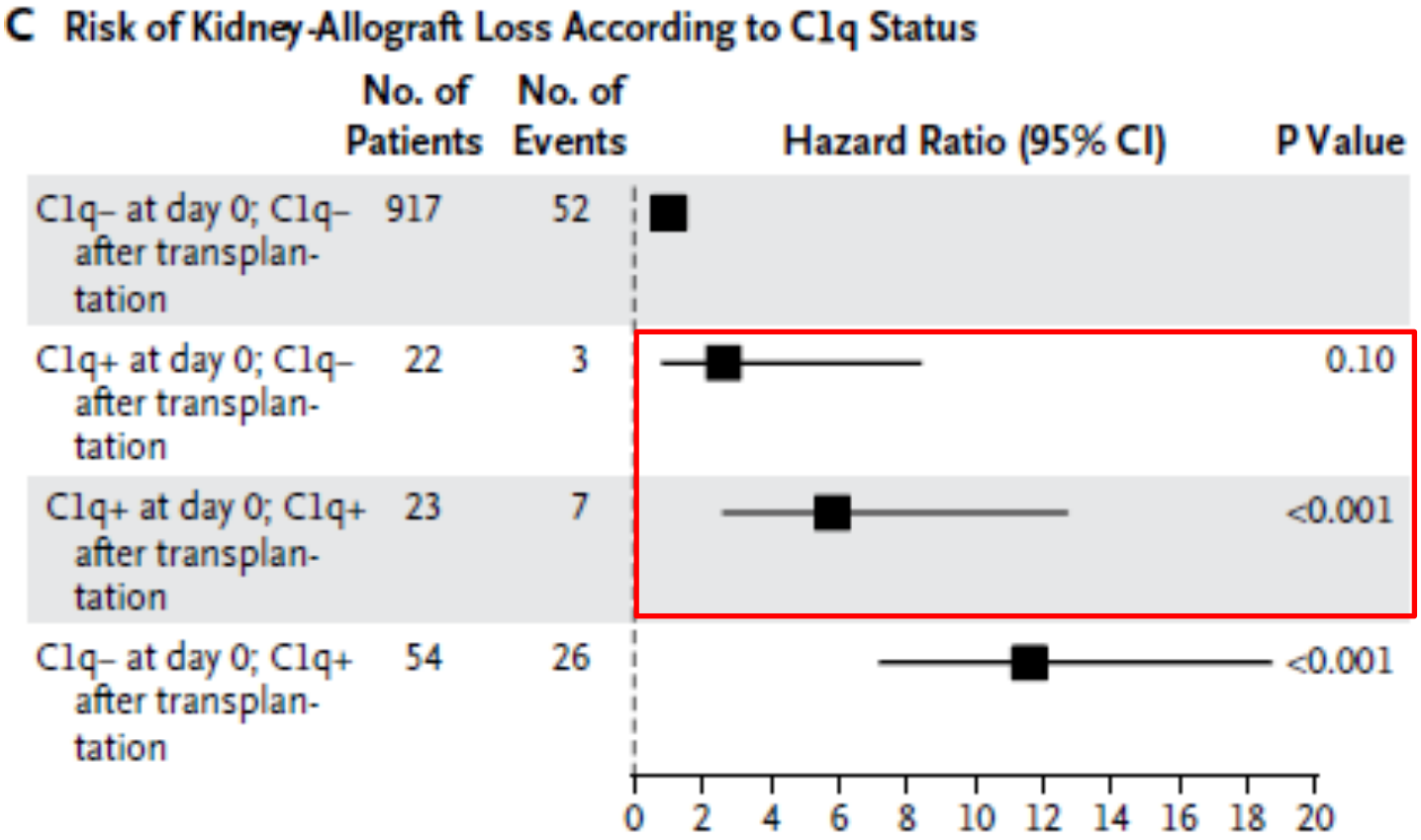
Kidney allograft survival according to DSA and C1q status





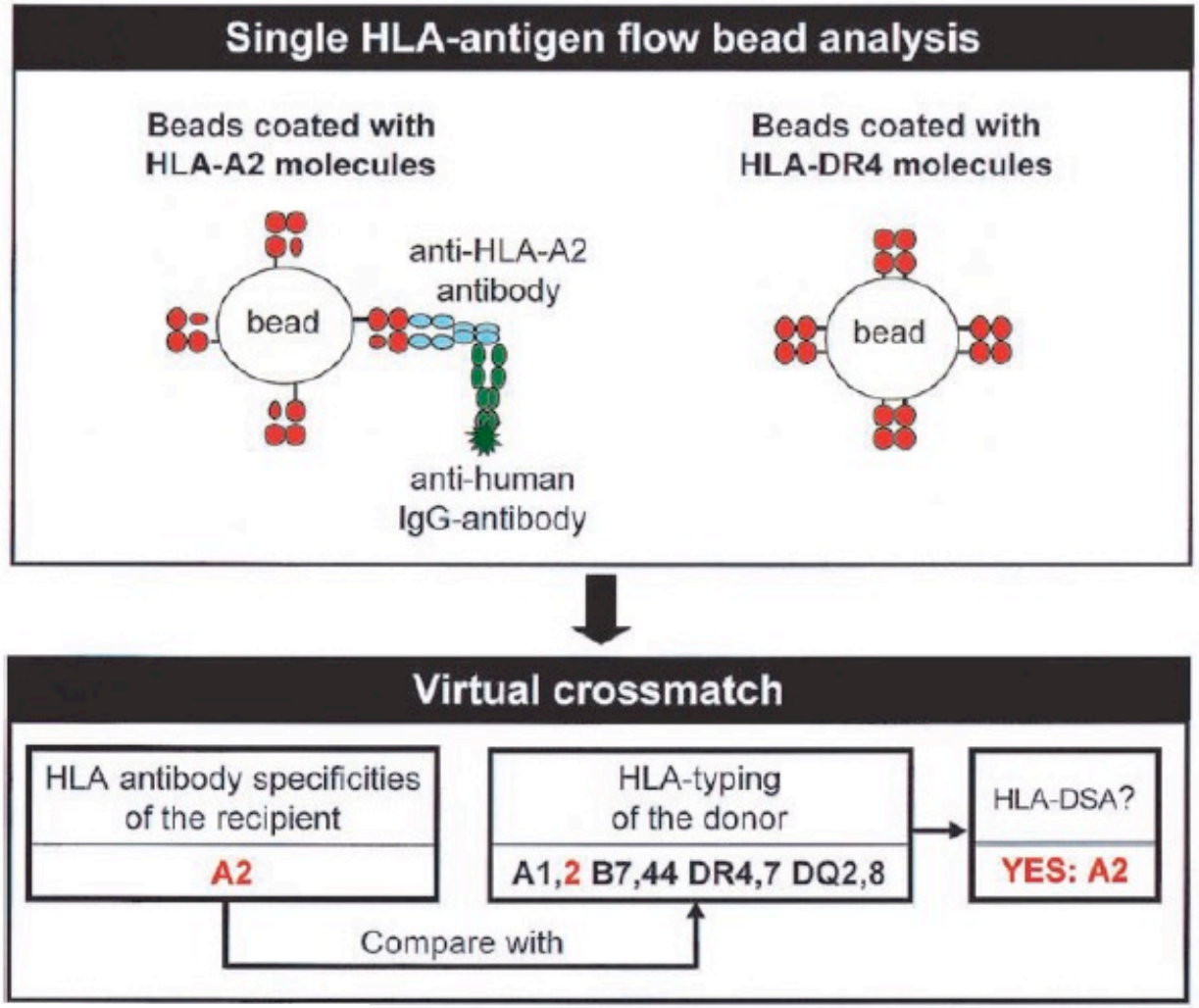
Loupy et al. N Engl J Med 2013;369:1215-1226

C1q+ pretransplant does not mean risk if postransplant status is negative



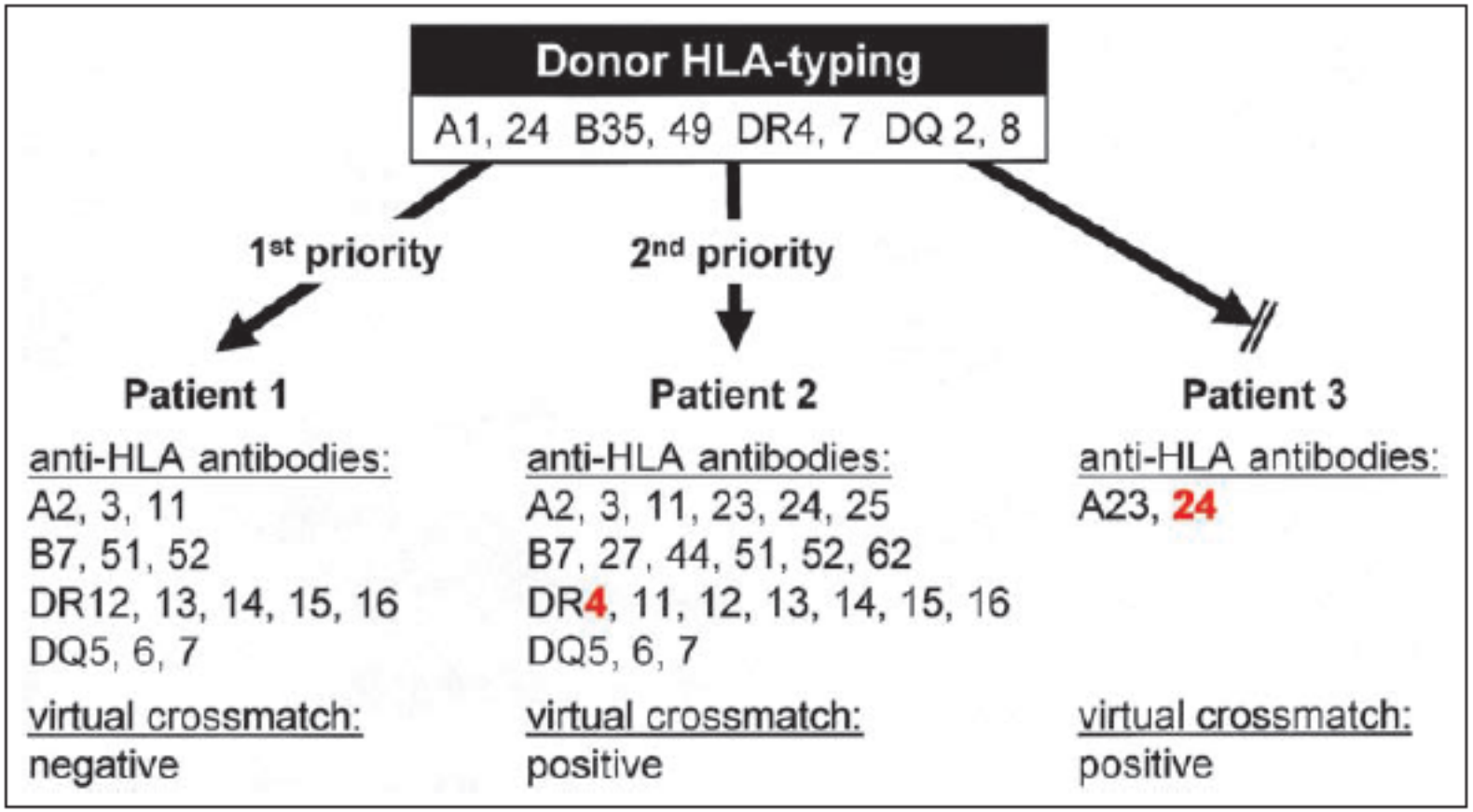
Loupy et al. N Engl J Med 2013;369:1215-1226

Koncept virtuálního crossmatch



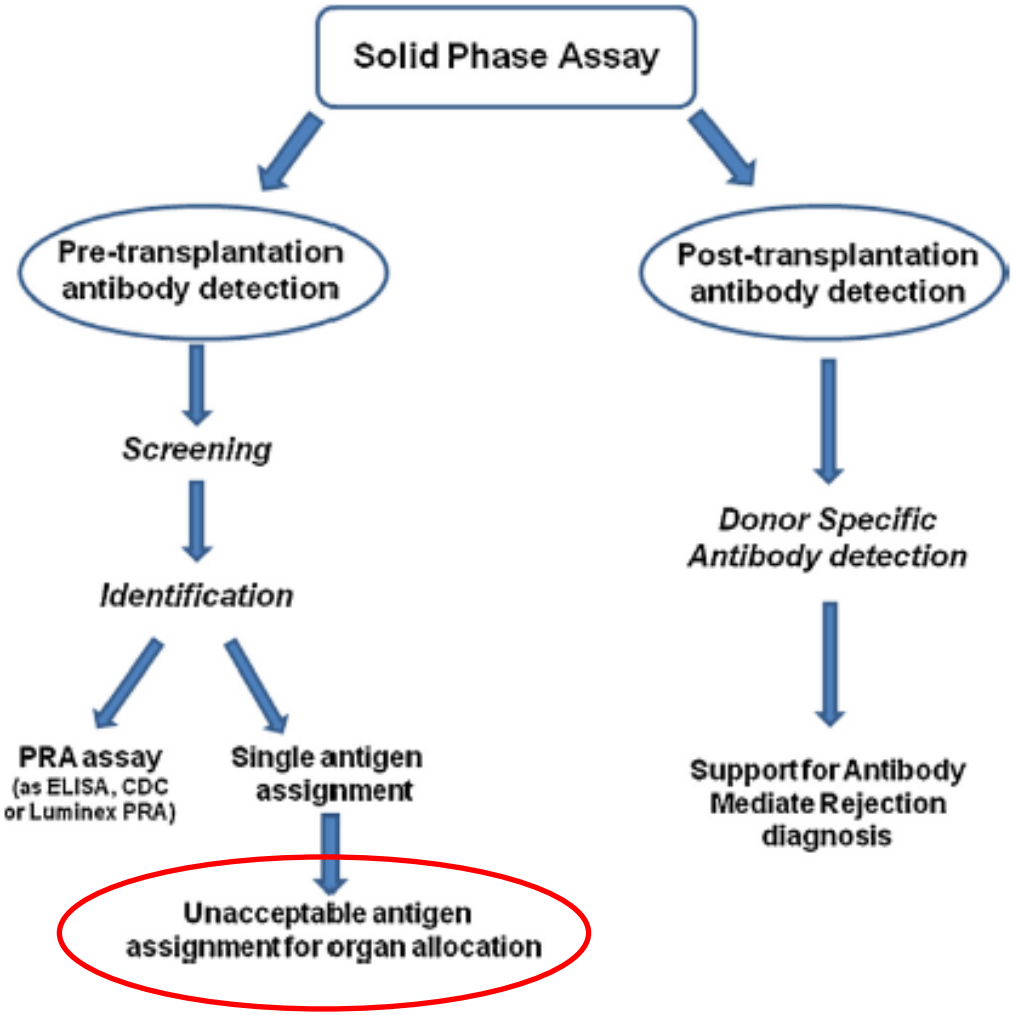
SWISS MED WKLY 2008;138(33–34):472–476

Virtuální crossmatch a alokace ledviny



SWISS MED WKLY 2008;138(33–34):472–476

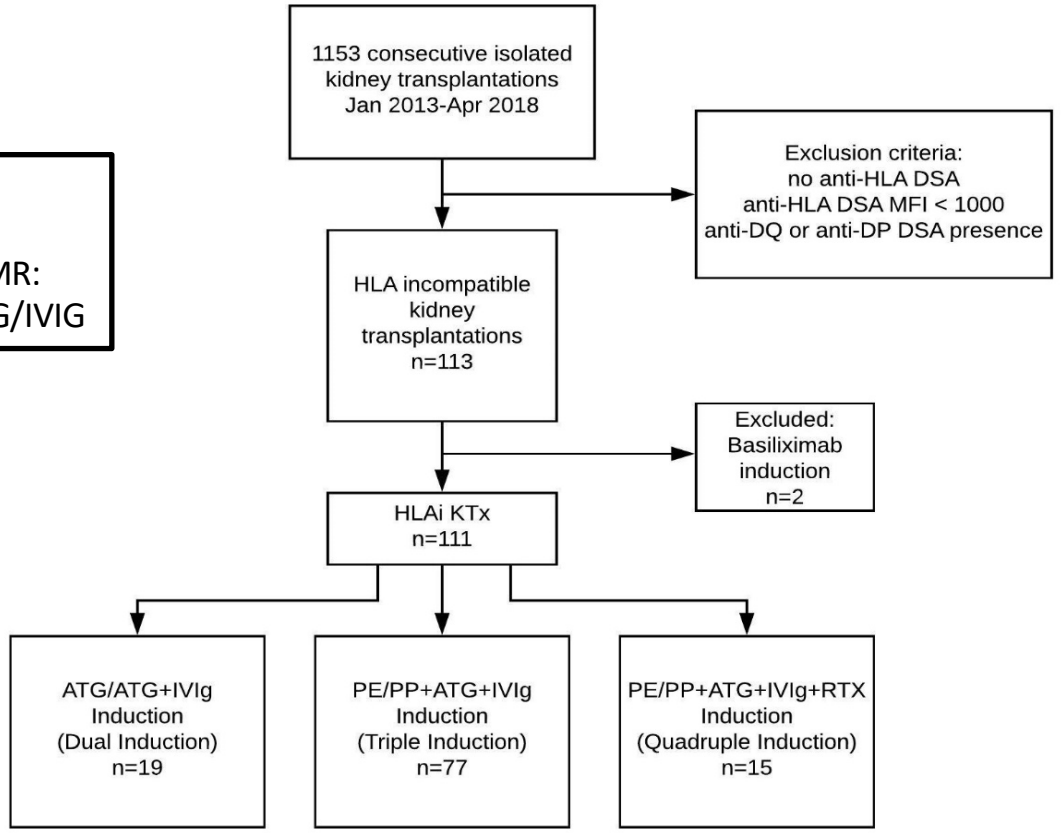
Využití detekce anti HLA protilátek před a po transplantaci ledviny



Immunology Letters 162 (2014) 181–187

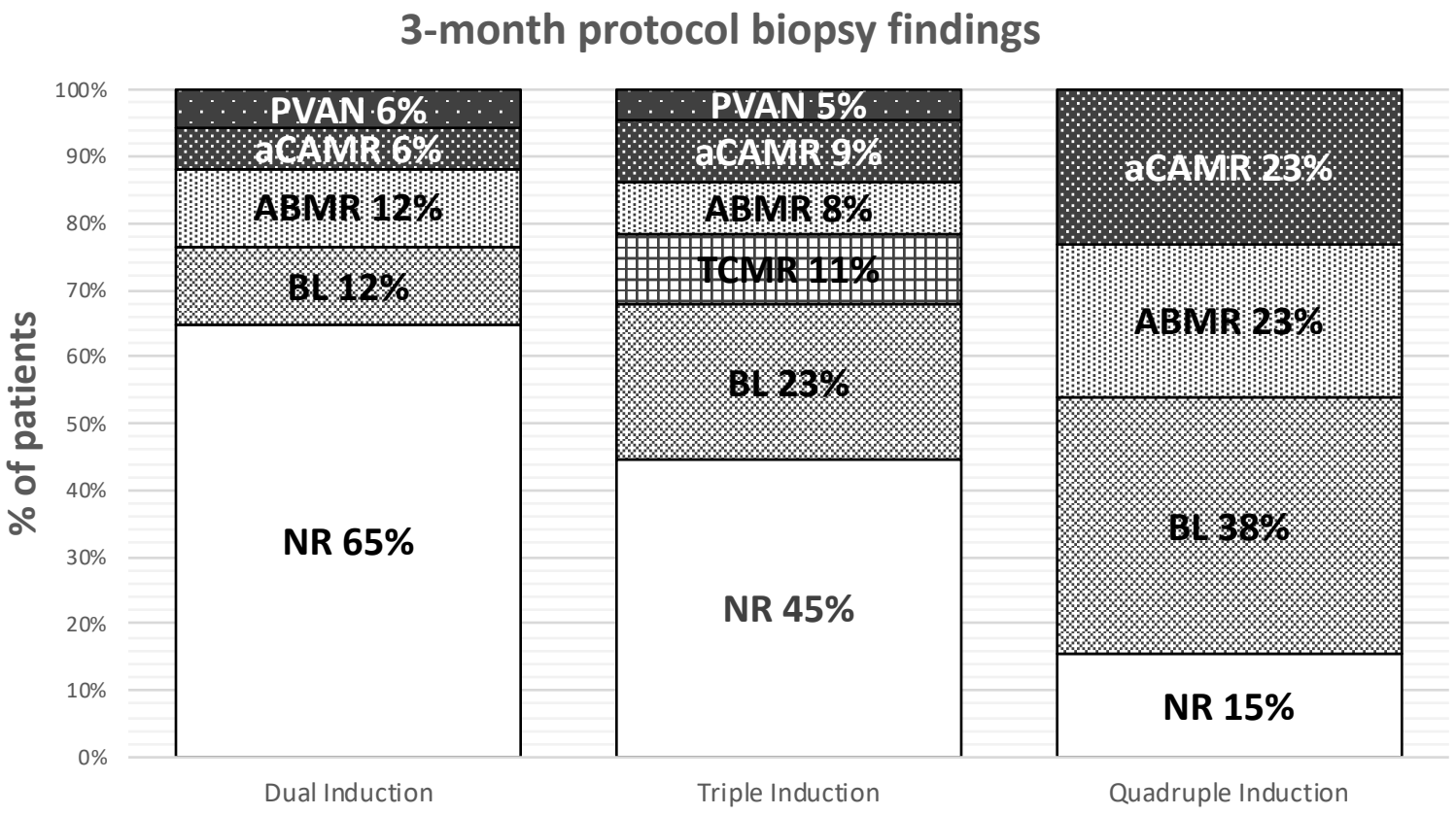
HLAi transplantation at IKEM: induction strategy according to DSA levels

DSA < 5 000 MFI:
PE/PP/rATG/IVIG
DSA < 10 000 MFI, 2nd, 3rd TX, history of AMR:
Induction with RTX, PE/PP/rATG/IVIG



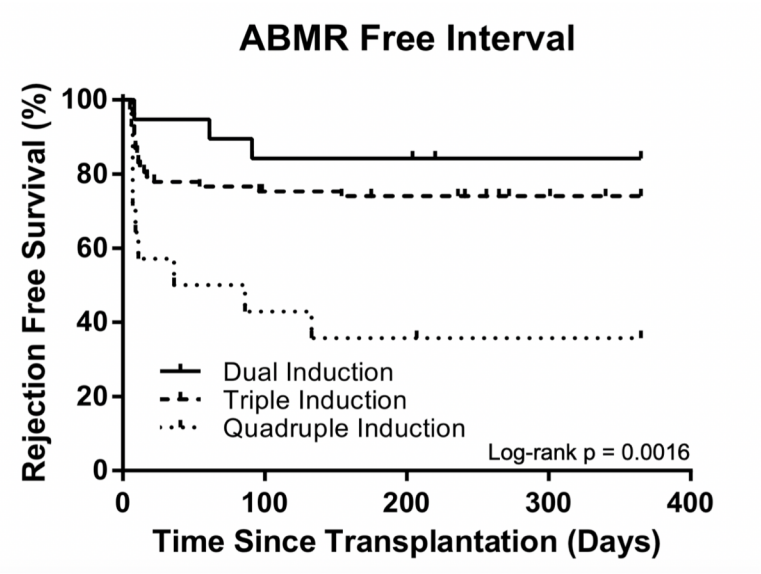
Osickova K. and Viklicky O., 2019, submitted

Patients at increased immunological risk experienced more ABMR at 3M biopsies

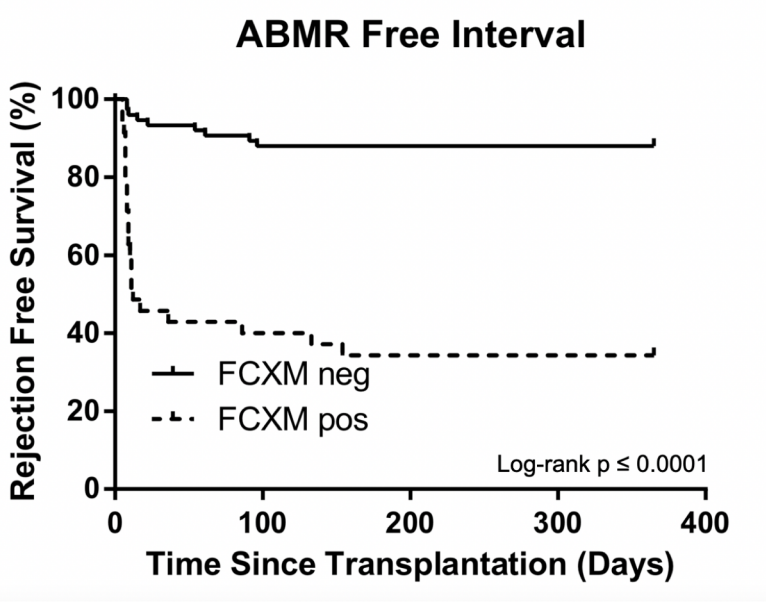


Osickova K. and Viklicky O., 2019, submitted

Pretransplant FCXM better than DSA in rejection prediction



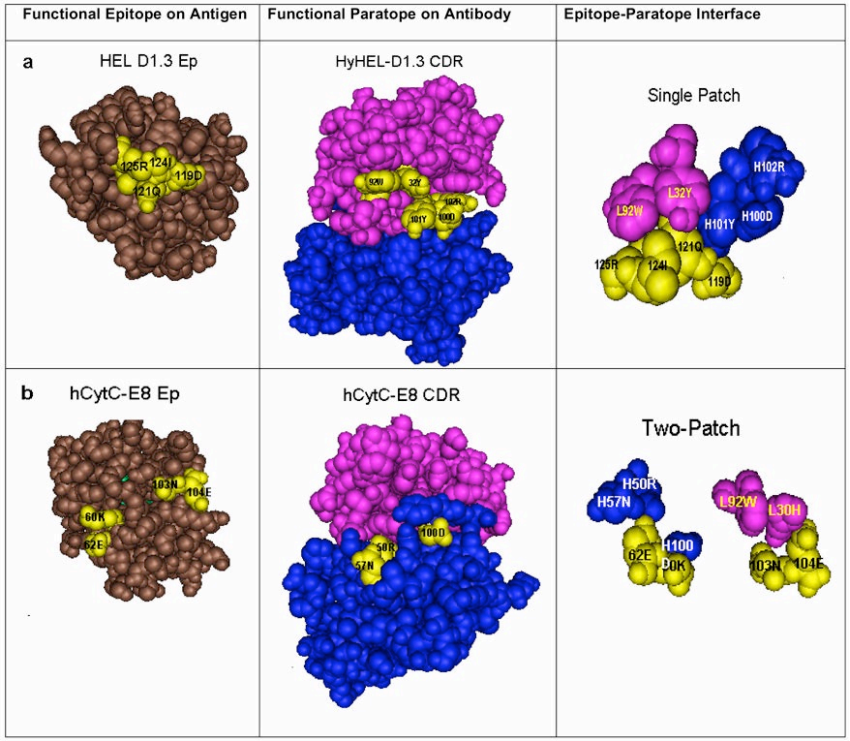
No. at Risk					
Dual Induction	19	17	17	15	14
Triple Induction	77	58	56	51	48
Quadruple Induction	15	7	6	5	4



No. at Risk					
FCXM neg	75	67	67	67	66
FCXM pos	36	15	13	13	12

Osickova K. and Viklicky O., 2019, submitted

Mismatched HLA antigens without foreign epitopes for recipient do not induce antibodies similarly to fully matched grafts



- HLAMatchmaker is a useful tool in the analysis of serum antibody reactivity of sensitized patients and the identification of potential donors with acceptable epitope mismatches
- Mismatch program to identify donors for highly sensitized patients in Eurotransplant and a similar program (Eurostam) is now being investigated for implementation in the European Union

R. Duquesnoy et al. 2014
F. Claas et al. 2014

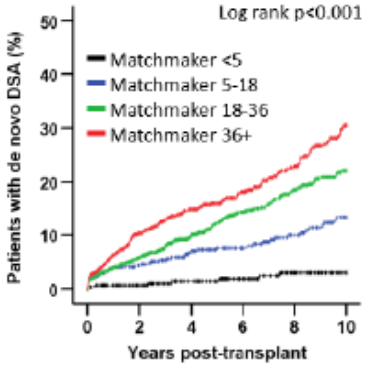
IK+E M

American Journal of Transplantation 2017; XX: 1–11
Wiley Periodicals Inc.

doi: 10.1111/ajt.14393

Donor–Recipient Matching Based on Predicted Indirectly Recognizable HLA Epitopes Independently Predicts the Incidence of *De Novo* Donor-Specific HLA Antibodies Following Renal Transplantation

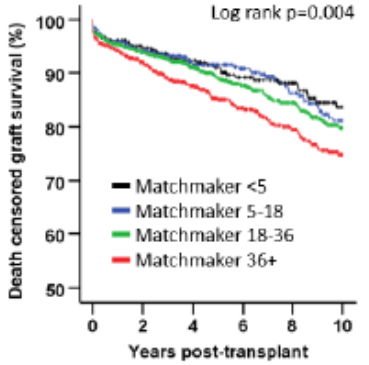
A



Number at risk

Years post-transplant	0	2	4	6	8	10
Matchmaker <5	323	280	236	193	145	101
Matchmaker 5-18	476	397	326	263	201	150
Matchmaker 18-36	1136	917	683	510	388	249
Matchmaker 36+	852	644	495	371	265	162

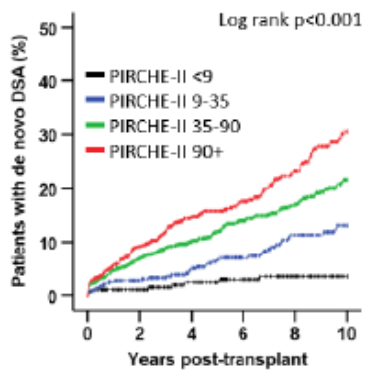
B



Number at risk

Years post-transplant	0	2	4	6	8	10
Matchmaker <5	323	268	222	179	136	90
Matchmaker 5-18	476	391	320	255	190	138
Matchmaker 18-36	1136	926	703	524	399	250
Matchmaker 36+	852	672	523	388	278	168

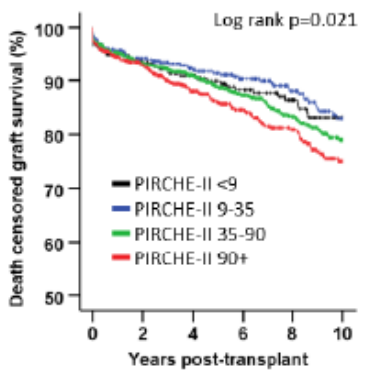
C



Number at risk

Years post-transplant	0	2	4	6	8	10
PIRCHE <9	285	246	203	168	126	87
PIRCHE 9-35	446	378	311	245	182	131
PIRCHE 35-90	1222	984	763	578	451	301
PIRCHE 90+	834	630	463	346	240	143

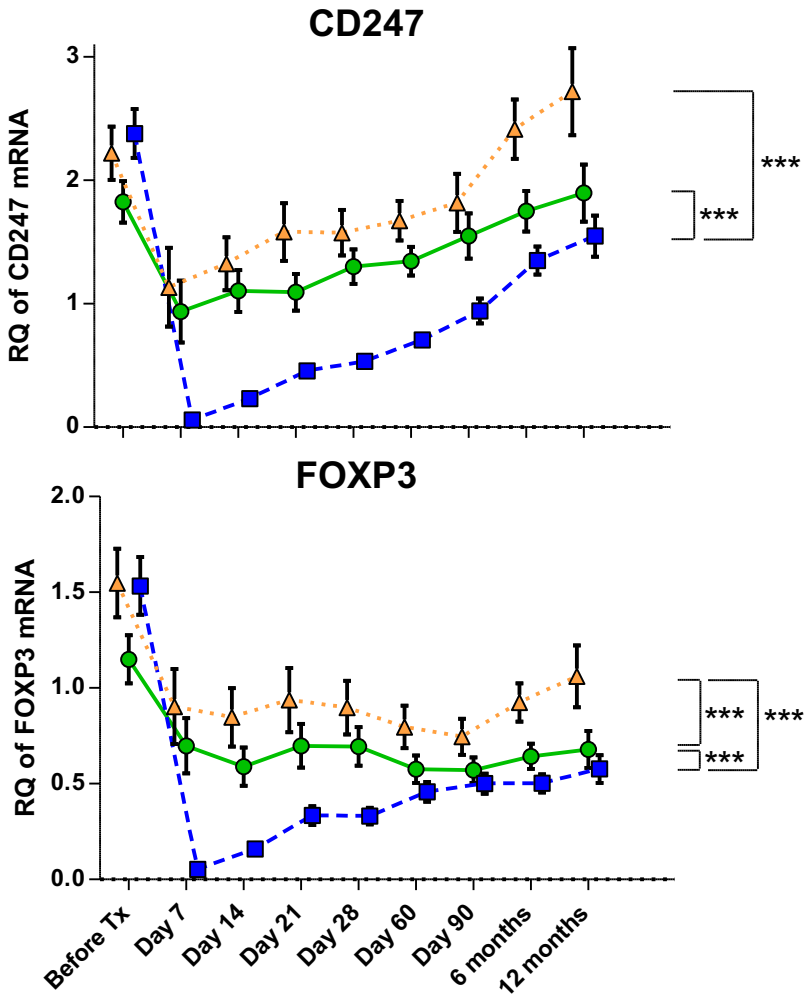
D

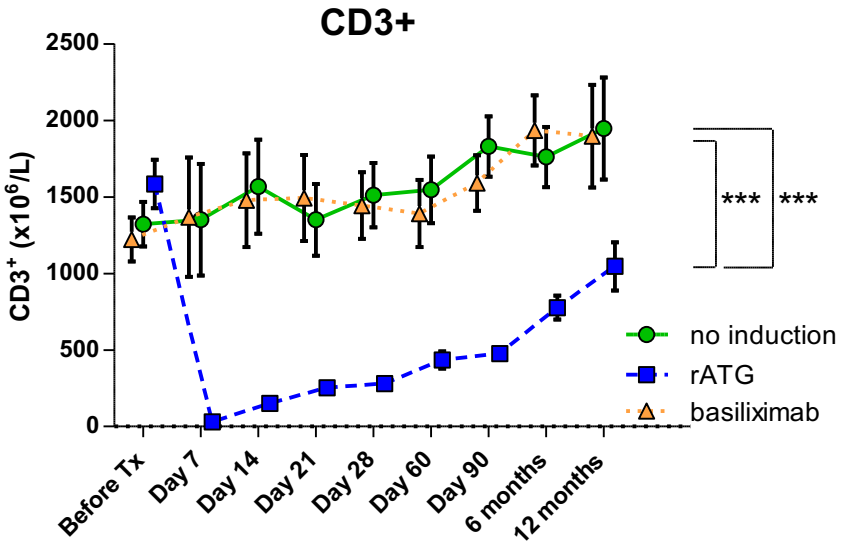


Number at risk

Years post-transplant	0	2	4	6	8	10
PIRCHE <9	285	234	190	155	116	77
PIRCHE 9-35	446	370	301	238	179	123
PIRCHE 35-90	1222	1001	787	590	450	299
PIRCHE 90+	834	652	490	363	258	147

Průtoková cytometrie a periferní transkripty k monitoraci po transplantaci

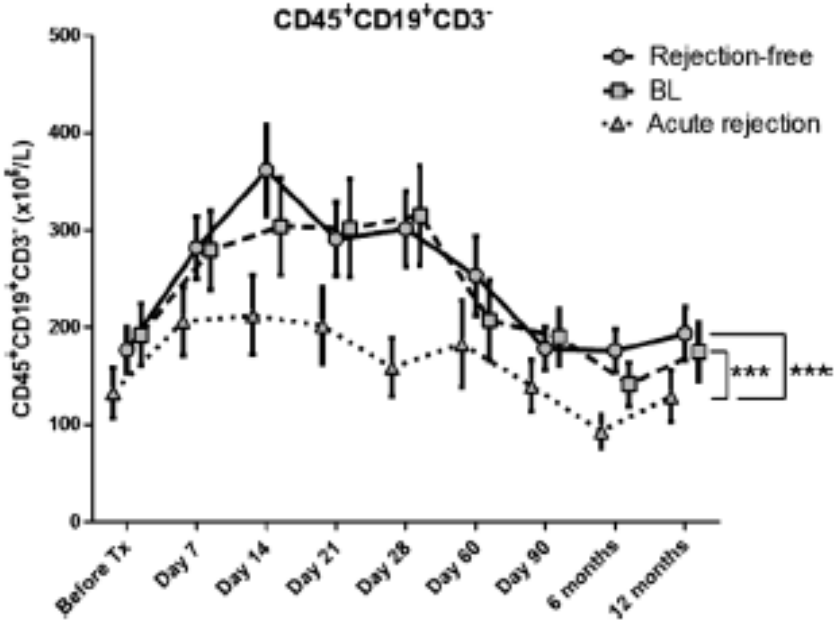


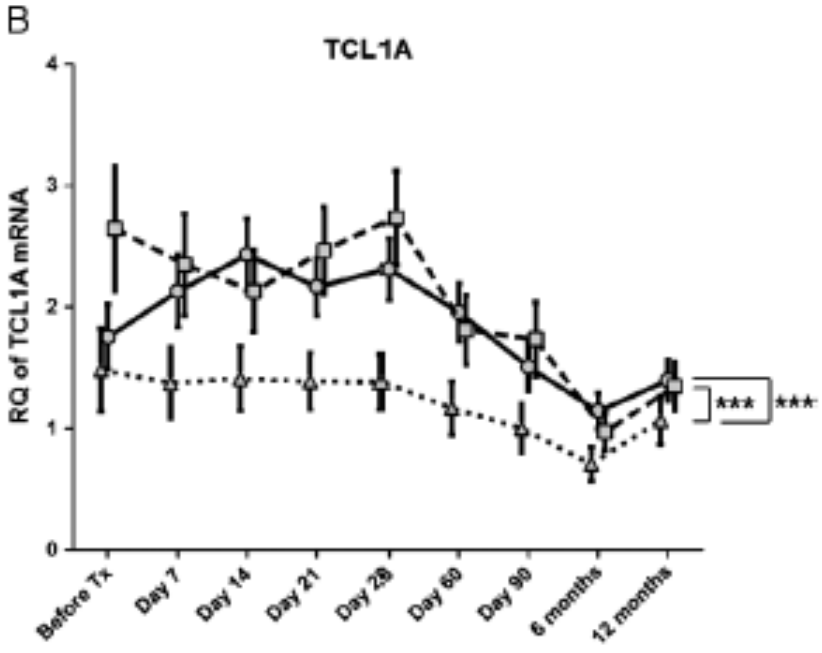


Kryštůfková a Viklický. Nephrol Dial Transplant. 2012 Jun;27(6):2576-82.

B-Cell-Related Biomarkers of Tolerance are Up-Regulated in Rejection-Free Kidney Transplant Recipients

N=69, TAC/MMF/St ± Induction





Viklicky O., et al. Transplantation 2013: 95:148-154.

17-gene set—the Kidney Solid Organ Response Test (kSORT)

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The kSORT Assay to Detect Renal Transplant Patients at High Risk for Acute Rejection: Results of the Multicenter AART Study

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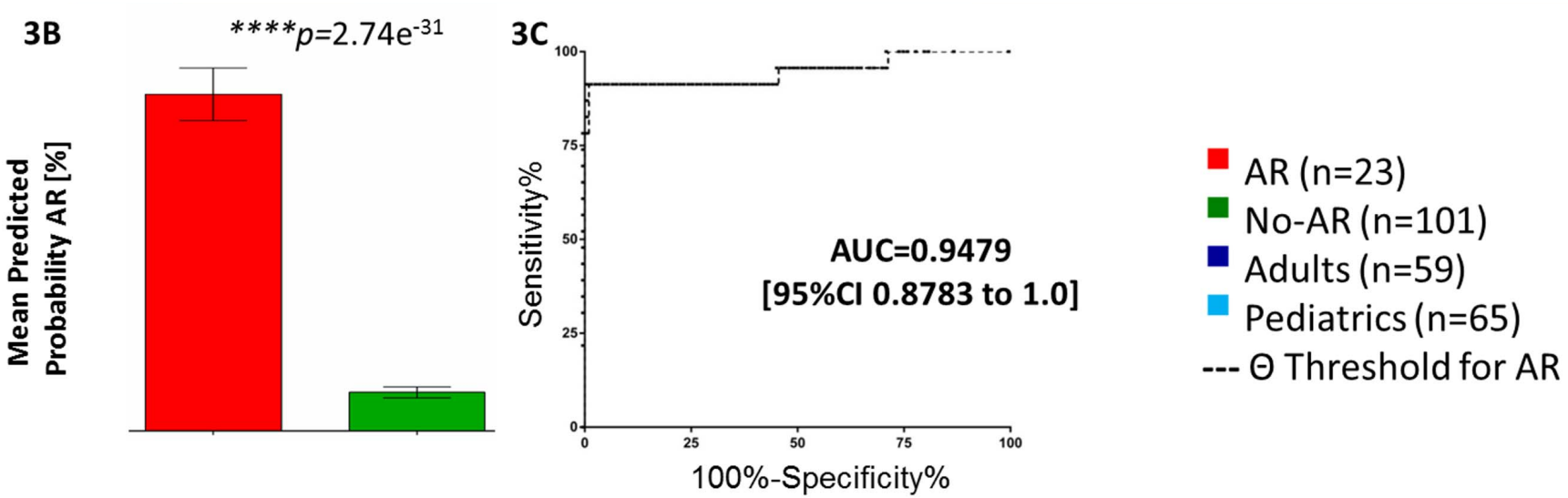
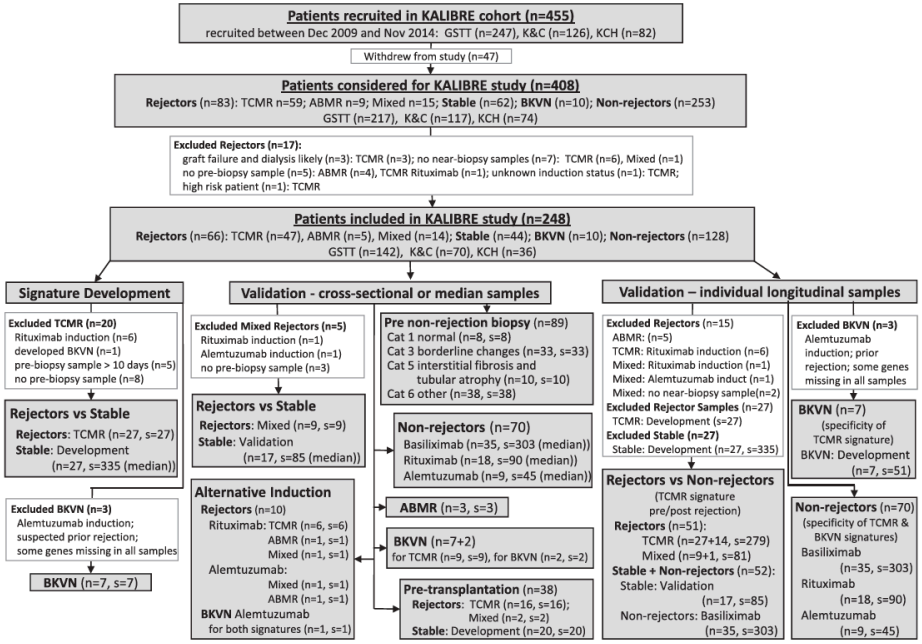


7-gene peripheral signature predicts TCMR in kidney transplantation (KALIBRE study)



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Development of a multivariable gene-expression signature targeting T-cell-mediated rejection in peripheral blood of kidney transplant recipients validated in cross-sectional and longitudinal samples

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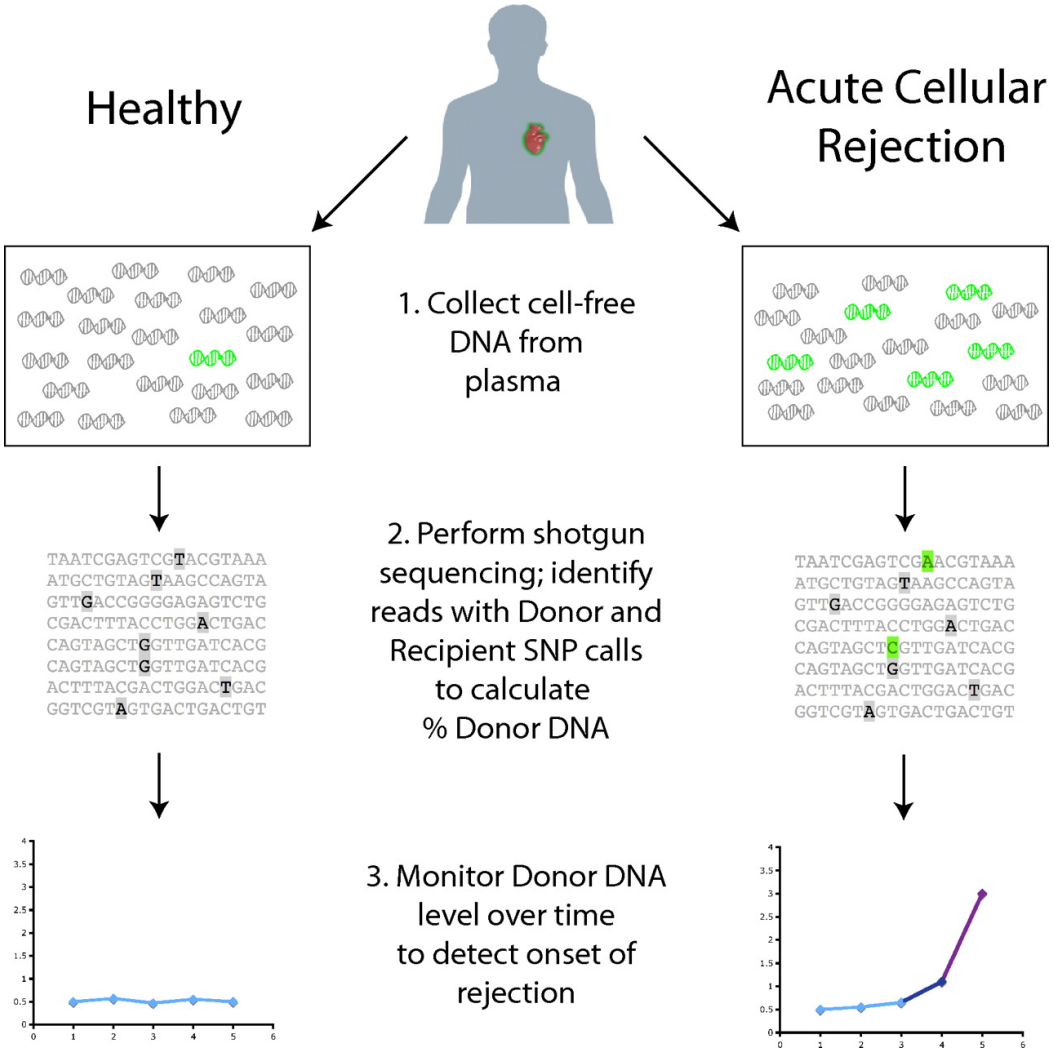
Gene-expression signatures.

Event	Genes	Data	AUC	Sens	Spec
TCMR	All genes	Training data	0.96 (0.92–1.00)	0·85	0·89
		Cross-validation	0.80 (0.75–0.83)	0.67 (0.59–0.70)	0.81 (0.71–0.88)
TCMR	*IFNG, IP-10, ITGA4, MARCH8, RORc, SEMA7A, WDR40A*	Training data	**0.95** (0.91–1.00)	0.85	0.93
		Cross-validation	**0.84** (0.77–0.88)	0.67 (0.59–0.74)	0.85 (0.75–0.89)
BKVN	All genes	Training data	0.93 (0.85–1.00)	0.71	0.93
		Cross-validation	0.68 (0.64–0.73)	0 (0–0.25)	0.91 (0.85–0.94)
BKVN	*IL-15, IL1R2, MARCH8, PDCD1, TGFB, WDR40A*	Training data	**0.95** (0.90–1.00)	0.71	0.91
		Cross-validation	**0.73** (0.66–0.80)	0.43 (0·29–0.57)	0.89 (0.83–0.91)

Donor derived cell-free DNA (dd-cf DNA)

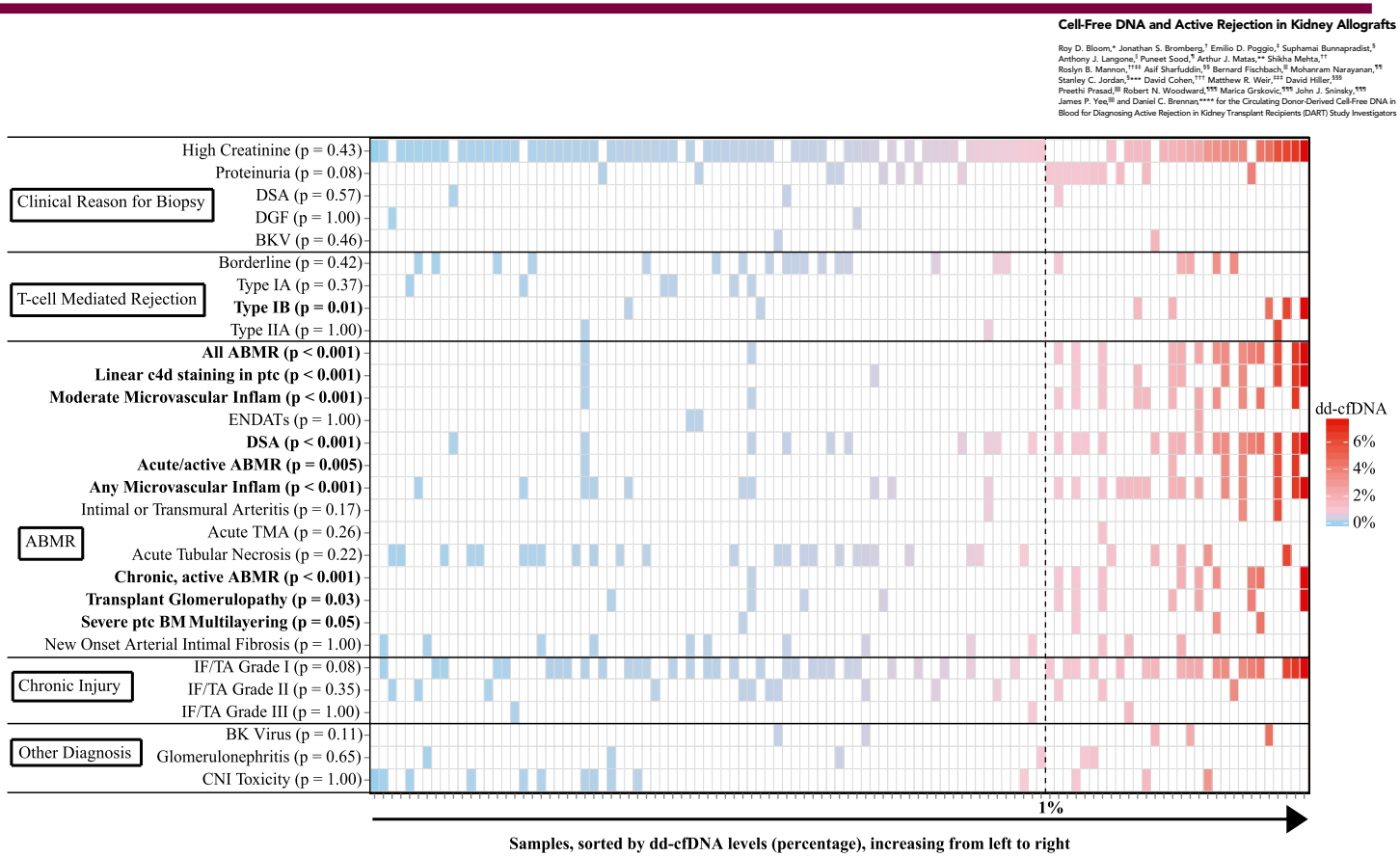


Thomas M. Snyder et al. PNAS 2011;108:15:6229-6234

IK+E M

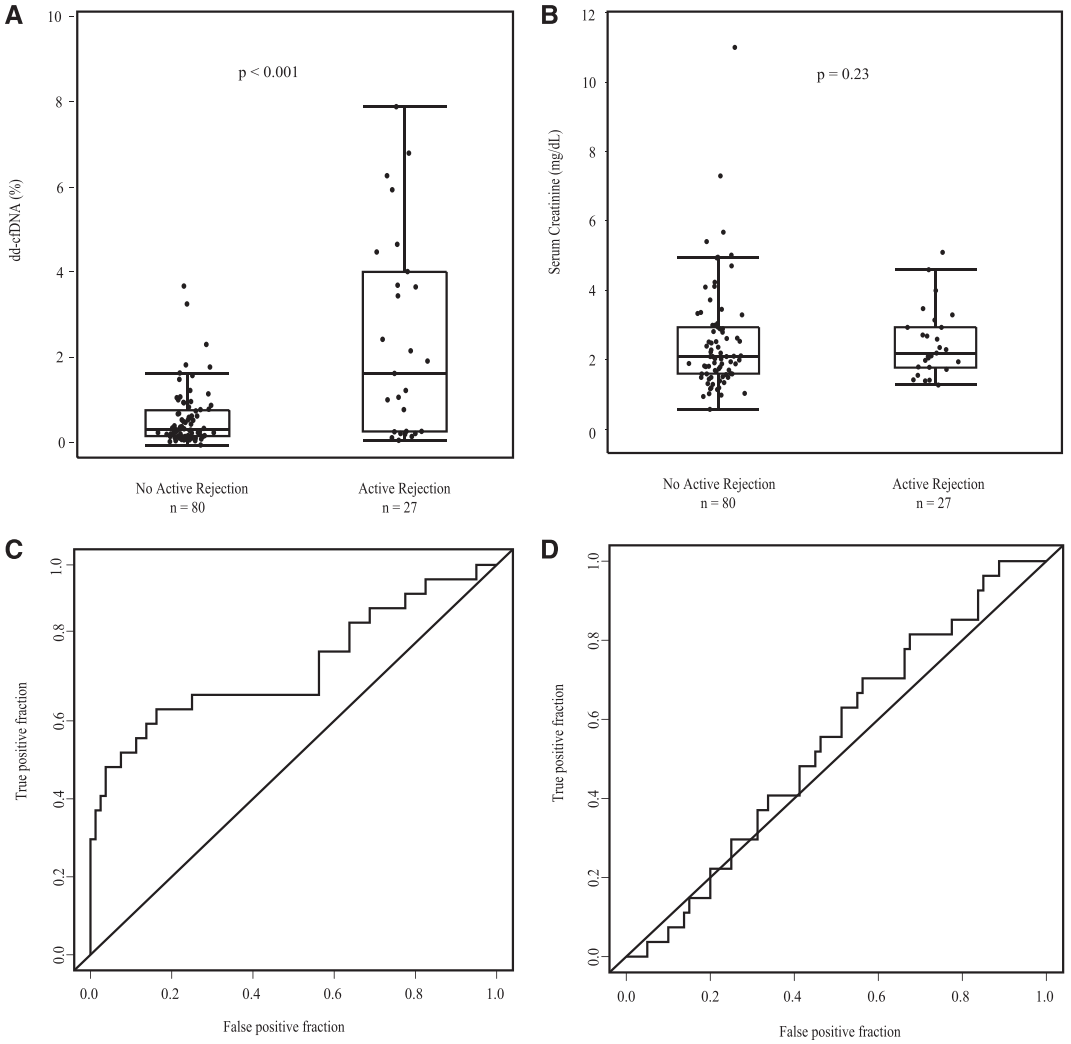
Cell-Free DNA as a marker of active rejection?

DART prospective study
n=102
107 biopsies



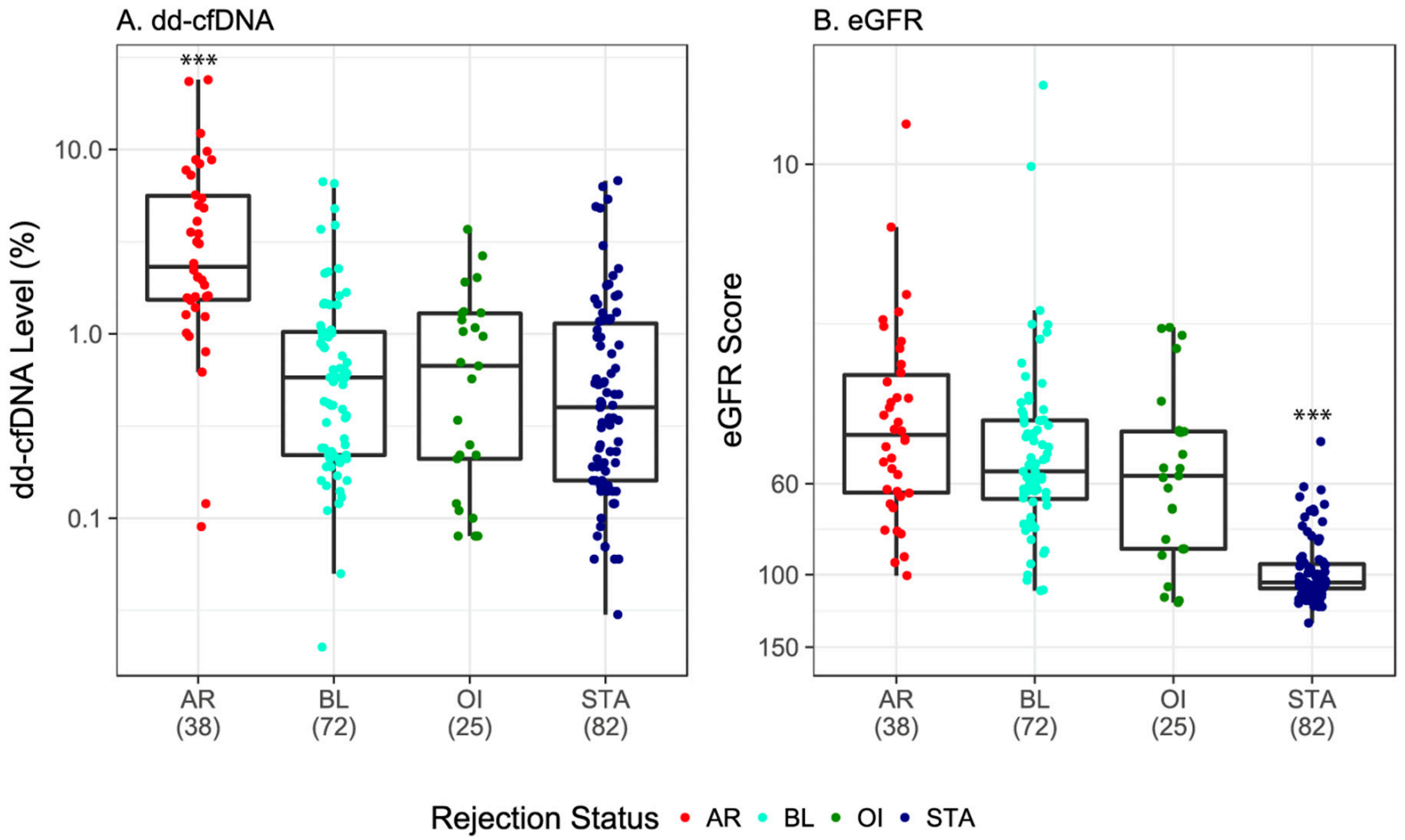
dd-cfDNA discriminates active rejection better than renal function

dd-cf DNA



Kreatinin

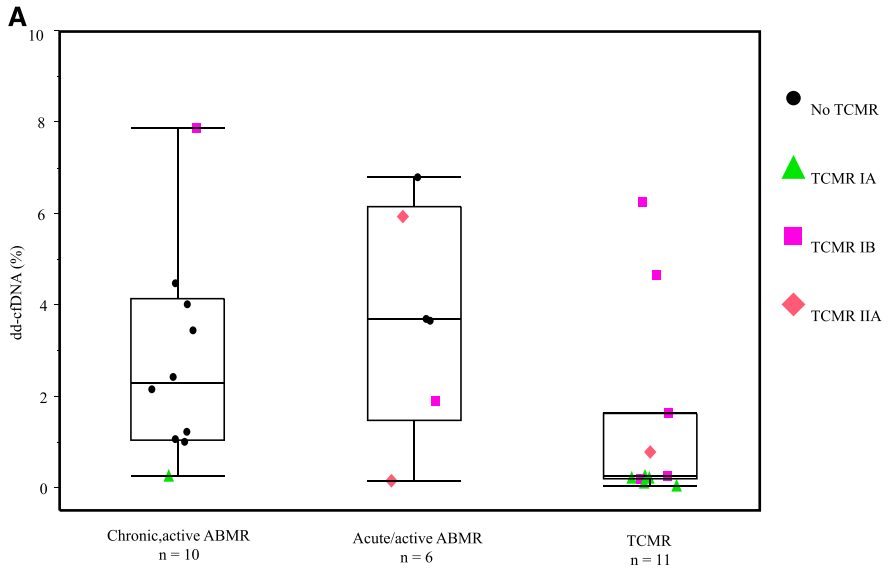
Discrimination of active rejection by dd-cfDNA versus eGFR

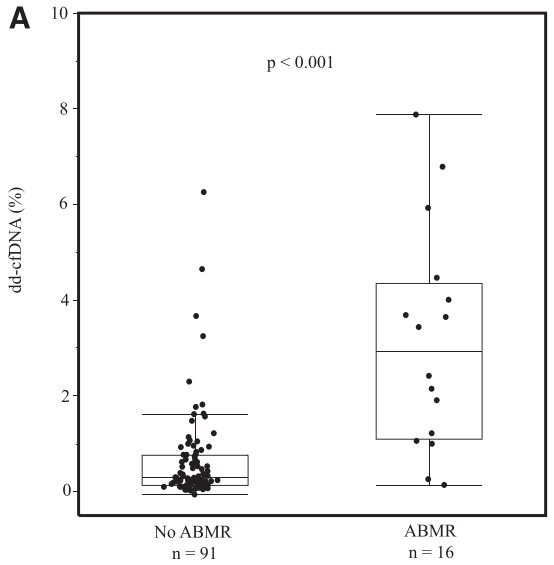


J. Clin. Med. **2019**, *8*, 19; doi:10.3390/jcm8010019

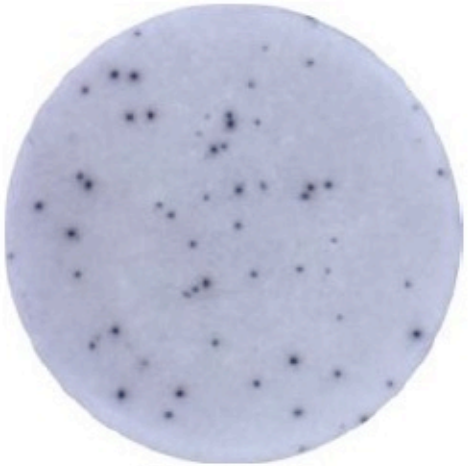
dd-cfDNA levels are higher in ABMR

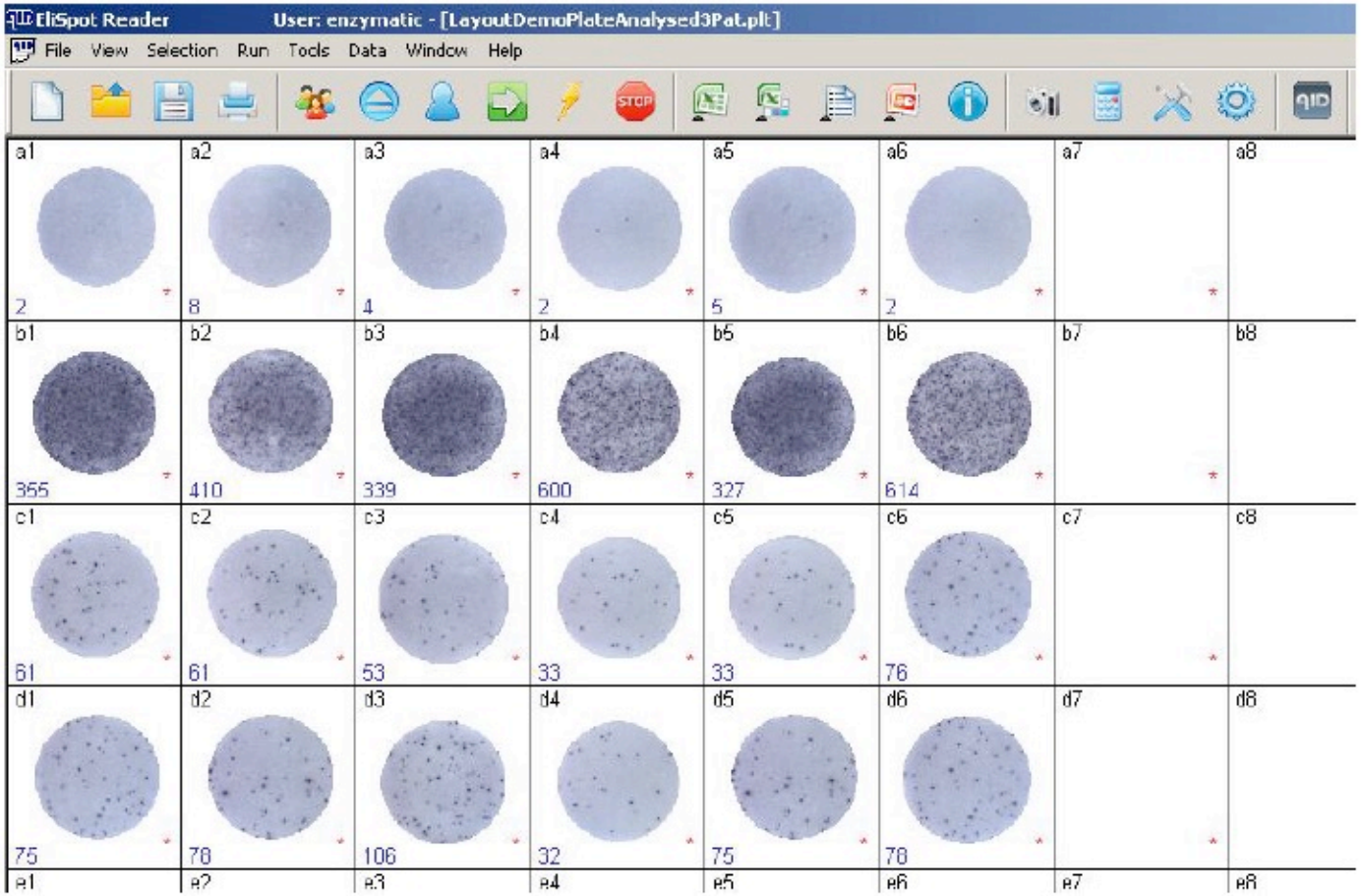
dd-cfDNA discriminates ABMR but not TCMR





ELISPOT: metoda k ověření přítomnosti paměťových lymfocytů





Dárcovská aloreaktivita koreluje se subklinickou rejekcí

Table 3 | The presence of 6-month d-s T-cell alloreactivity is associated with acute cellular subclinical rejection (TCSCR) in 6-month protocol biopsies

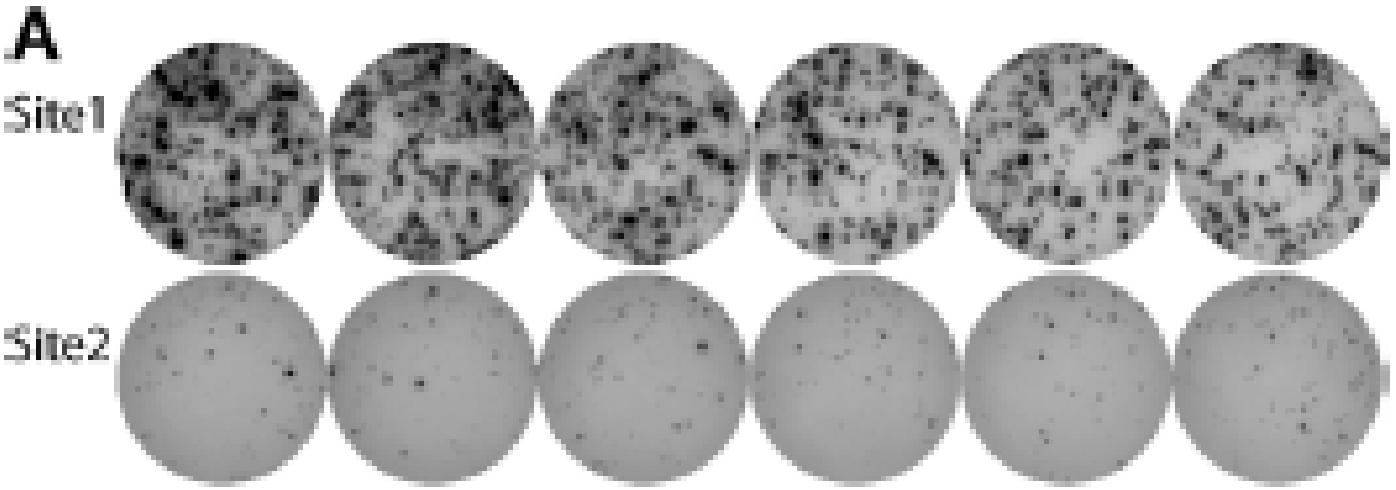
	6-Month d-s IFN-γ Elispot		
TCSCR	NEG	POS	$P<0.001$
No	32 (91.4%)	3 (27.3%)	
Yes	3 (8.6%)	8 (72.7%)	
Total	35 (100%)	11 (100%)	46

Kidney International (2013) **84,** 1226–1236;

IKEM

IFNγ ELISPOT vyšetření bez SOP

Previous ELISPOT data suffers from not standardized methodology: impossible to compare results from different centers



American Journal of Transplantation 2013; 13: 1871–1879
Wiley Periodicals Inc.

doi: 10.1002/ajt.12286

Brief Communication

Standardization and Cross Validation of Alloreactive IFNγ ELISPOT Assays Within the Clinical Trials in Organ Transplantation Consortium

Ashoor I. et al. Am J Transplant. 2013 Jul;13(7):1871-9

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ORIGINAL ARTICLE

Urinary-Cell mRNA Profile and Acute Cellular Rejection in Kidney Allografts

Manikkam Suthanthiran, M.D., Joseph E. Schwartz, Ph.D., Ruchuang Ding, M.D., Michael Abecassis, M.D., Darshana Dadhania, M.D., Benjamin Samstein, M.D., Stuart J. Knechtle, M.D., John Friedewald, M.D., Yolanda T. Becker, M.D., Vijay K. Sharma, Ph.D., Nikki M. Williams, B.S., Christina S. Chang, B.S., Christine Hoang, B.S., Thangamani Muthukumar, M.D., Phyllis August, M.D., M.P.H., Karen S. Keslar, M.S., Robert L. Fairchild, Ph.D., Donald E. Hricik, M.D., Peter S. Heeger, M.D., Leiya Han, M.D., M.P.H., Jun Liu, Ph.D., Michael Riggs, Ph.D., M.P.H., David N. Ikle, Ph.D., Nancy D. Bridges, M.D., and Abraham Shaked, M.D., Ph.D., for the Clinical Trials in Organ Transplantation 04 (CTOT-04) Study Investigators

N Engl J Med 2013;369:20-31.

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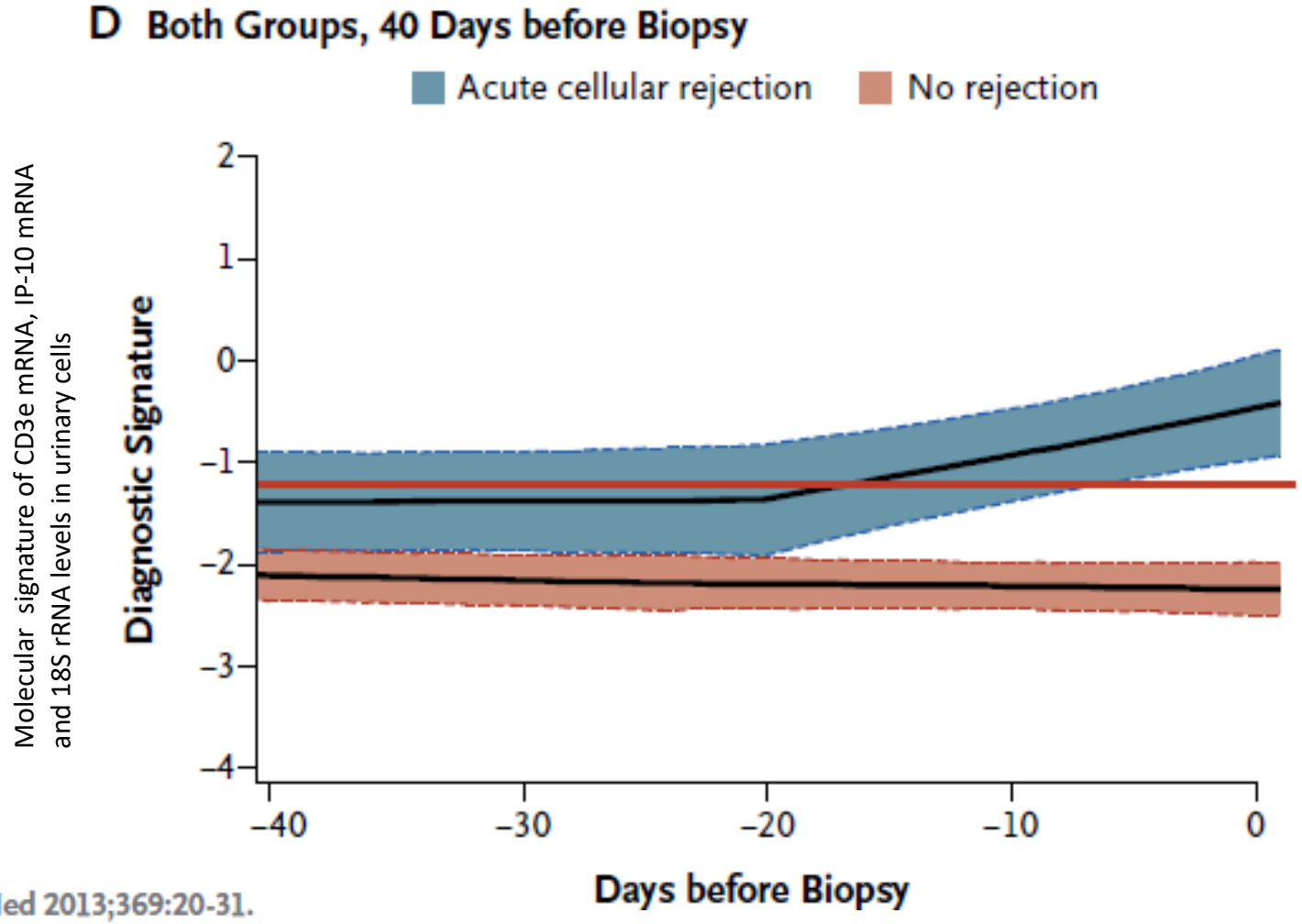
Vyšetření transkriptů z moče predikuje rejekci

Prospective study of 430 patients monitored, 4000 urinary samples, validation cohort



N Engl J Med 2013;369:20-31.

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Imunologické metody použitelné po transplantaci

- LUMINEX (anti HLA, DSA)
- FACS (subpopulace T a B buněk, FCXM)
- RT-PCR (mRNAs exprese – transkripty, dd-cfDNA)
- Microarray (MMDx – molekulární patologie)
- ELISA (IP-10, KIM.. nemají prediktivní roli)
- IFN-gamma ELISPOT (zřejmě slepá cesta)

Možnosti predikce imunologické odpovědi po transplantaci

- Výskyt akutní rejekce se v poslední době pohybuje do 12%, v polovině případů jde o humorální rejekci
- Hlavním důvodem ztráty štěpu v dlouhodobém sledování je chronická humorální rejekce
- Předtransplantační predikce humorální odpovědi je stále založena na „starých“ testech PRA, CDC, FACS CM ale rovněž na virtuálním crossmatch (anti HLA skríning v době zařazení do čekací listiny). Definice neakceptovatelných antigenů respektive neakceptovatelné síly protilátek zůstává nejasně definována, zdá se, že budoucnost má definice akceptovatelných epitopů!
- Z nových testů má největší šanci na klinické uplatnění test prokazující přítomnost dd cf DNA – zatím jen k monitoraci humorální odpověd

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