Non-invasive detection of renal allograph rejection

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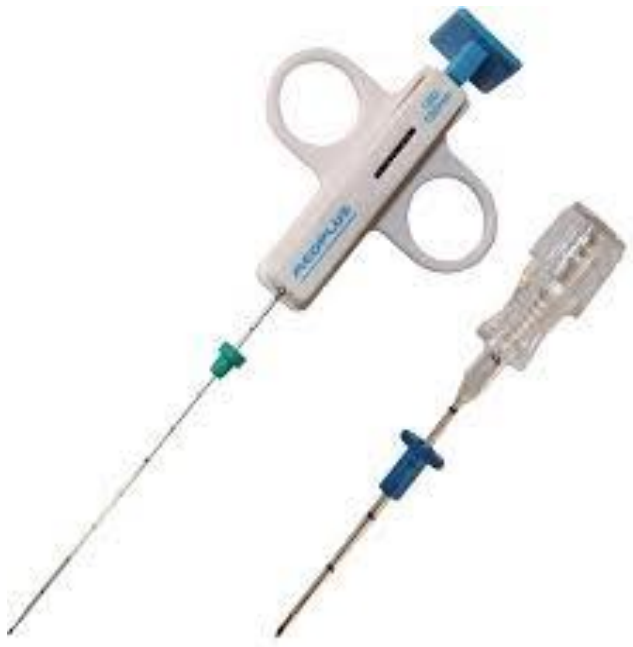
Case scenarios

- Case 1:
  - 26 yr old, transplanted 8 years ago. Creatinine 78micromol/L, stable and well. Newly married, pregnant, abrupt rise in creatinine to 150 micromol/L at 16 weeks pregnancy
- Case 2:
  - 42 yr old, transplanted 4 weeks ago. Slow, progressive climb in creatinine from 130 micromol/L by 50micromol/L per week. Recent drop in Hb by 3 g/dl, platelets 74000
- Case 3:
  - 31 yr old, transplanted 4 years ago. Coincidental discovery of doubling of serum creatinine from 250 micromol/L on routine 3-monthly visit.

Biopsy still the gold standard

- Graft dysfunction: a very wide differential…
- Always keep time from transplant in mind
- Other than rejection: The OBVIOUS ones
  - Obstruction
  - CNI toxicity
  - Graft Pyelonephritis
- Histological mimickers
  - Interstitial nephritis (drug- or infection related)
  - Viruses: CMV & BK
  - PTLD

What makes us hesitate?



- 1. Complications
  - *Whittier, CKJ October 2018*
  - *Peters B et al, Acta Radiologica 2014*
- 2.Unfit patients
- 3. Incomplete answers, time delays

Kidney biopsy: Banff…

- Category 1 Normal or non-specific changes
- Category 2 Antibody mediated changes Active antibody mediated rejection
- Chronic active antibody mediated rejection
- Chronic (inactive) antibody mediated rejection
- C4d staining without evidence of rejection
- Category 3 Suspicious for T cell mediated rejection (borderline)
- Category 4 T cell mediated rejection Acute T cell mediated rejection (Grade I-III)
- Chronic active T cell mediated rejection (Grade I-II)
- Category 5 Polyomavirus nephropathy Grade I

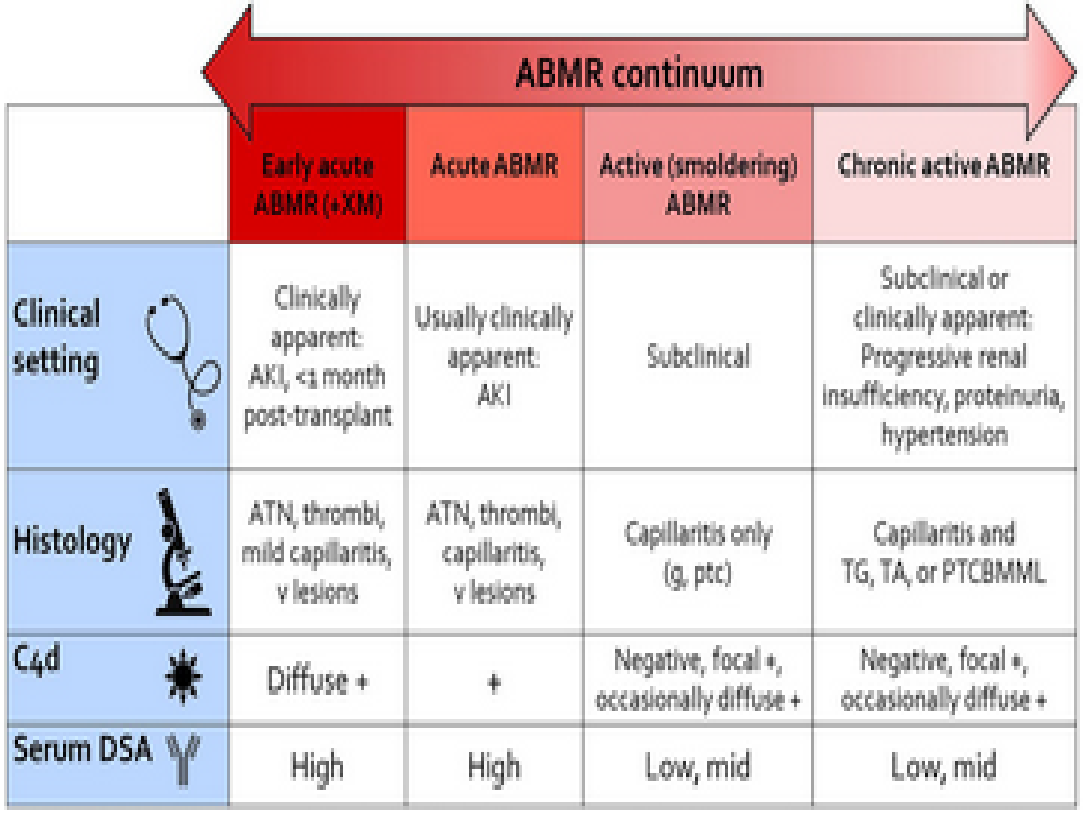


FIGURE 3 ABMR continuum. This schematic provides a reference for thinking about the continuum of "pure ABMR" in kidney transplant recipients with preformed DSA, as detailed in this article. Not included in the figure is combined ABMR and T cell mediated rejection in patients with *de novo* DSA and under-immunosuppression (iatrogenic or due to nonadherence). AKI, acute kidney injury; ATN, acute necrosis/injury; g, glomerulitis; ptc, peritubular capillaritis; v lesions, Banff vascular lesions (endothelialitis, fibrinoid necrosis of vessels); TG, transplant glomerulopathy; TA, transplant arteriopathy; PTCBMML, peritubular capillary basement membrane multilayering (by electron microscopy); +XM, positive crossmatch.

Cornell, LD: Histological features of antibody-mediated rejection: the Banff classification and beyond. Front. Immunol. 27 September 2021

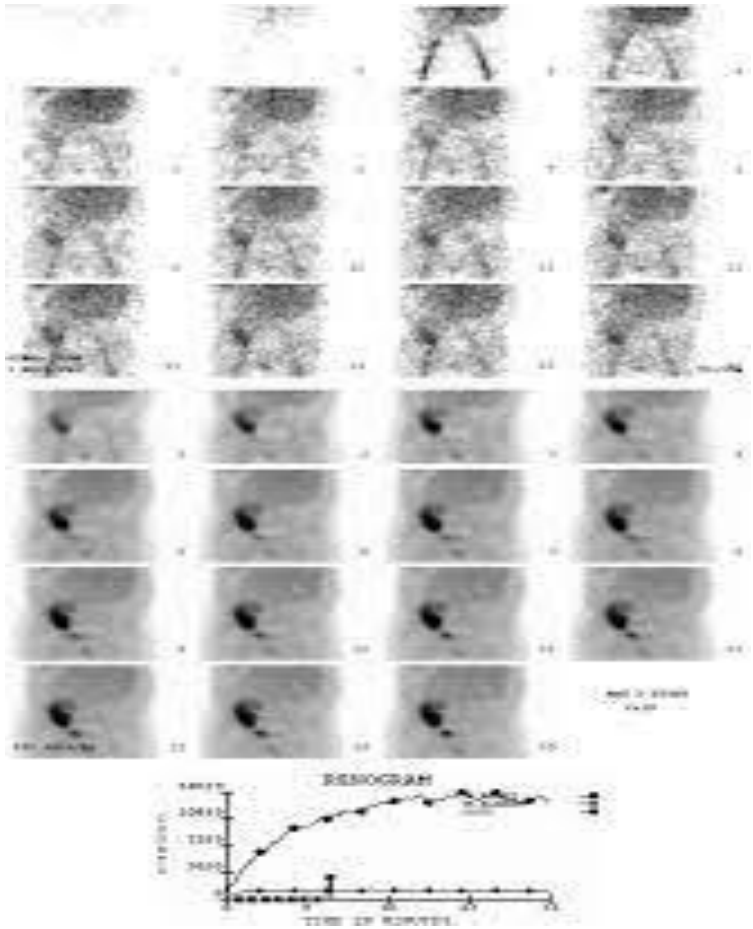
Traditional non-invasive methods: ("Old-school")

- Bio-markers:
  - Creatinine
  - Proteinuria
  - DSA's
- Imaging:
  - Ultrasound
  - Nuclear renography

Traditional non-invasive methods: ("Old-school")

- Imaging:
  - Ultrasound: increased graft size, loss of CMD, hypoechoic pyramids, decreased echogenicity…
    - Non-specific
    - Also tells us about obstruction, fluid collections, vascular patency
  - Doppler Resistance indices? Keep in mind the wide list of causes of a raised RI!

Nuclear Renography



- 3 Phases: perfusion , concentration & excretion:
- Early baseline
- Comparative studies
- MAG3 previously favored, now DTPA
- Can assist with diagnosis of thrombosis, obstruction or urine leak
- Diagnosis “suggestive of”, & Can’t differ between ABMR & cellular rejection
- CAVEAT: CNI toxicity can jinx all

Volkan-Salanci B, Erbas B. Imaging in renal transplants: an update. Semin Nucl Med 51:364-379 , 2021

Anything new from Nuclear Medicine?

- **Nuclear renography:**

Multiparameter texture analysis differentiates ATN from AR

(sensitivity: 88%, specificity 92.3%)

Concept: allograft rejection causes tissue changes. These changes can affect the texture of a kidney image.

Ardakani AA, et al: Scintigraphic texture analysis for assessment of renal allograft function. Pol J Radiol 83:e1-e10, 2018

- **Radiolabeled Leucocyte scintigraphy**

Several studies showing potential benefit (early rejection vs ATN, 81% sensitivity)

Grabner's T-lymphocyte rat study not verified in humans

- **F18 – FDG PET scanning**

Activated leucocytes need energy!

Uptake independent of renal fx.

Biomarkers: 1.BLOOD

Plenty markers!

-Simon T, Am J Transplant 2003: serial perforin & granzyme B gene expression in peripheral blood

-**Aquino-Dias, KI 2008:** Parameters associated w FOXp3 gene expression in delayed graft function of benefit

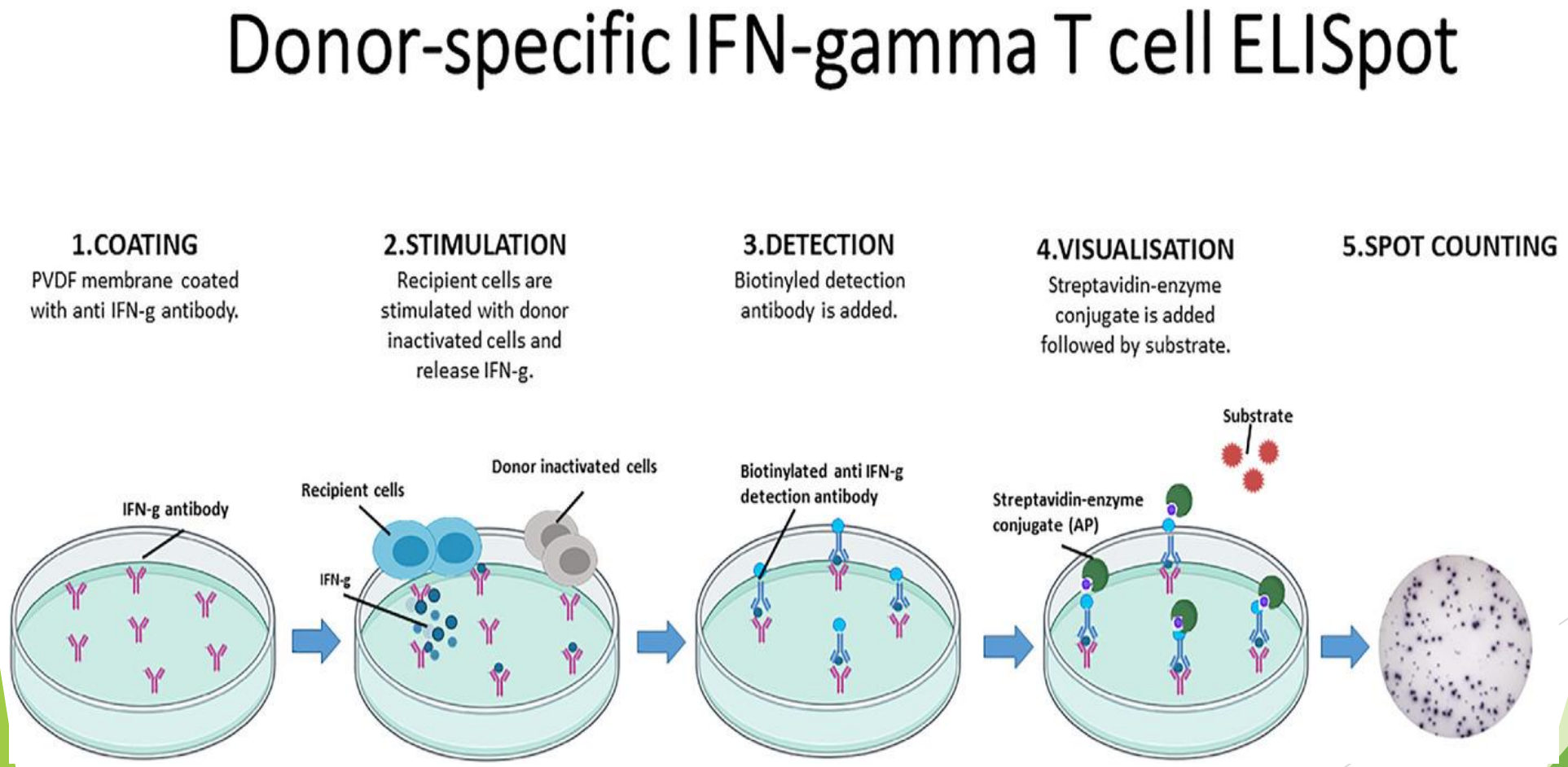
-Gunter OP, Transplantation 2009: 160 genes differentially expressed in peripheral blood samples of pts with biopsy confirmed acute rejection

-Kurian SM, PloS1 2009: Gene expression profiles reveal over 2400 genes for mild CAN, and over 700 for moderate/severe CAN.

-Matz M, Transplantation 2016: combined measurement of microRNA arrays may help to better identify T-cell mediated vascular rejection

ETC ETC ETC

What if we could do functional cell-based immune monitoring?



ELIspot (continued)

Germanova E et al. ELIspot assay and prediction of organ transplant rejection. Int J Immunogenet 2022 Feb 49(1)

interferon (IFN)-gamma enzyme-linked immunospot assay

- Increased frequency of AR, poorer graft fx at 12 months
- HLA mismatching= +ElIspot, +Acute rejection
- no association between +ELIspot pre-transplant and AR in patients who got ATG

MUCH criticism of single-center studies: lack of uniformity

- Montero (meta analysis, 2019): sensitivity 64% specificity 65% for predicting AR
- Negative predictive value>90% in low risk patients
- Suboptimal for clinical use, but may improve in combination w other biomarkers

“kidney recipients with high numbers of T and B memory cells may not always develop rejection, which could be due to high tolerogenic immunity”

- HLA-specific Ig G B cell & donor-specific B cell ELIspot:
  - Currently a clinical dead-end

Kidney Solid Organ Response Test (kSORT)

- Method:

- Advantages (AART trial, Plos Med, November 2014)
  - Predict pts at risk (Sens 92%, spec 93%)
  - Predicted rejection in 60% up to 3 months prior
  - Identified 12 of 16 cases of subclinical rejection
  - Combined with ELIspot: improves accuracy for subclinical AR , and distinguishing between T-cell- & ABMR

- Subsequent studies FAILED TO VALIDATE its utility for detection of AR in the 1st year under real-world conditions
- Commercialization program unclear (Immucor DX)

Donor-derived cell-free DNA

- Idea “stolen” from fetal medicine
- CONCEPT: Plasma levels of dd-cfDNA released into the bloodstream by dead cells in the injured allograft
- -elevated in patients with acute rejection
- Cut-off determined at 1%

- Overall, PPV 61% NPV 81%

- Correlates w biopsy findings of AR BUT can’t distinguish between T-cell & ABMR (although median dd-cfDNA higher for ABMR)
- Commercially: Plasma Allosure & Prospera – available, busy w registry studies

Biomarkers: 2. URINE

- **PROTEINS**
  - chemokine (C-X-C motif) ligands 9 and 10 (CXCL9 and CXCL10)
  - CXCL9: T-cell mediated rejection (PPV 68% NPV92%)
  - CXCL10: ABMR
  - CTOT1 study: PPV low, NPV better – best application to determine pts at LOW risk for T-cell mediated rejection (drug weaning!) BUT increased levels also in BK virus nephropathy
- **Messenger RNA's**
  - kidney allograft may function as an "in vivo flow cytometer"
  - Single-center studies: perforin, granzyme-B, IFN-inducible protein 10
  - CTOT4 (2013) : very promising 3-gene signature for determining TCMR, and distinguishing it from ABMR
  - Can detect weeks before clinical evidence of graft dysfunction, BUT extensive degradation of mRNA is a limitation.

Biomarkers: 2. URINE (continued)

- Urine proteomics/peptidomics: Currently a quagmire.

- Urine microRNA's:
  - Small ribonucleotides, regulating gene expression.
  - Initial study compared stable Tx pts , those with UTI, & acute graft dysfunction
  - miR-210 and 10-b downregulated in acute rejection, miR-210 at low level also predicted poorer graft fx at 1 year.
  - Maluf DG (KI 2014): subset of MiRNA's found in patients with interstitial fibrosis & tubular atrophy, compared to those with normal graft function, can be used to monitor & project worsening graft function.

Summary

- Limited accuracy, lowish PPV's, often NPV more of value
  - Many tests have a role in diagnosis of only one specific part of the puzzle
- Costly, unpractical
  - Under which circumstances, & in what order?
  - Naesens M, et al. A Practical Guide to the Clinical Implementation of Biomarkers for Subclinical Rejection Following Kidney Transplantation. Transplantation, April 2020
- May guide therapy? One day, but not yet.

The evolution of Banff… Invasive molecular markers