Analysis of RNA Transcripts by the Molecular Microscope Diagnostic System (MMDx) Can Direct Management after Indication Kidney Transplant Biopsy

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Abstract

Establishing the cause of kidney allograft dysfunction is often reliant on biopsy appearances. Despite biennial refinements in the Banff classification there remain challenging issues such as ‘borderline’ for T Cell-Mediated Rejection (TCMR). Recently the Banff classification has introduced the potential for molecular assessment of the troubled kidney transplant. We report two cases of graft dysfunction in which the clinical decision making was potentiated by the Molecular Microscope® Diagnostic System (MMDx), both at indication and follow up biopsy. In the first case a patient with ‘borderline TCMR’ changes by traditional methods was shown to have ‘severe’ TCMR by MMDx and when re-biopsied because the creatinine did not improve MMDx demonstrated resolution of the molecular signals of rejection. Conversely the second case was diagnosed with Banff 1a TCMR by traditional methods, but the MMDx gene transcript analysis showed no rejection. Follow up biopsy showed no rejection by either traditional methods or MMDx and blinded re-reporting of the first biopsy disagreed with the initial histopathology report and agreed with MMDx. In both cases The Molecular Microscope offered certainty when traditional pathology was uncertain and if relied upon has the potential to better guide therapeutic interventions.

Abbreviations

TCMR: T Cell-Mediated Rejection; MMDx: Molecular Microscope® Diagnostic System; APKD: Adult Polycystic Kidney Disease; PTLD: Post-Transplant Lymphoproliferative Disease; DSA: Donor-Specific Antibodies; RNA: Ribonucleic Acid; ABMR: Antibody-Mediated Rejection

Introduction

Traditional assessment of kidney allograft dysfunction relies on a combination of laboratory tests, radiological assessment and histopathology. Conventional histopathology follows the recommendations of the Banff working group which meets every two years to revise diagnostic criteria [1]. The Banff classification is a formidable body of work but it is an iterative, consensus-based process which is constantly being refined. Application of Banff criteria to kidney allograft pathology must inevitably be open to a degree of intra- and inter-observer variation [2]. Various studies have investigated the concept that it is possible to investigate the molecular basis of graft dysfunction by identifying gene transcripts which are altered in disease states, this can be performed in blood [3] or tissue [4,5]. The 2013 Banff iteration [6] incorporated a working group to establish the role of molecular analysis of kidney transplant biopsies and included ‘Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury’ in the diagnostic criteria for Antibody-Mediated Rejection (ABMR).

The INTERCOMEX study demonstrated the utility of the Molecular Microscope® Diagnostic System (MMDx) which uses microarrays in a 3 mm core of cortical kidney transplant biopsy to assess rejection states in real time [7]. The INTERCOMEX investigators concluded that that ‘MMDx more frequently agreed with clinical judgment (87%) than did histology (80%)’.

We report two cases of graft dysfunction which help demonstrate the utility of MMDx in clinical practice.
Case Presentation

Case 1

A 65 year old female with End Stage Renal Failure (ESRF) due to autosomal dominant Adult Polycystic Kidney Disease (APKD) received a live unrelated kidney transplant from her husband in August 2008. The kidney was 2-1-0 ABDR HLA mismatch and maintenance immunosuppression was with Tacrolimus, Mycophenolate Mofetil and Prednisolone until May 2017 when she was diagnosed with Post-Transplant Lymphoproliferative Disease (PTLD). Consequent to the PTLD diagnosis the mycophenolate was discontinued and she was treated with Rituximab, Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone (R-CHOP). She achieved remission from the PTLD and continued with stable graft function (serum creatinine 85 µmol/L [0.96 mg/dL]). Maintenance immunosuppression continued thereafter with Tacrolimus and prednisolone 5 mg daily, without an antiproliferative agent. Tacrolimus levels (12 h trough) were most recently 4.5 ng/mL and had not been below 4 ng/mL. At a routine clinic visit the serum creatinine was found to have risen to 135 µmol/L (1.53 mg/dL). The patient had no symptoms. Repeat blood test confirmed the graft dysfunction and an USS was normal. She had no Donor Specific Antibodies (DSA) to donor HLA A, B, Cw, DR or DQ antigens. There was a denovo anti-DP antibody but donor DP type was unknown because donor DP typing was not routine prior to her transplant in 2009. An Ultrasound scan of the transplant kidney was normal.

An ultrasound guided biopsy was performed with a spring loaded 18G trucut biopsy needle. Two cores were taken as is standard practice. With the patient’s prior consent the smaller of the two cores was placed immediately in to RNA later and shipped at room temperature to the Kashi lab, Portland, OR, USA for testing with the Molecular Microscope Diagnostic System (MMDx). The larger (13 mm core) was sent for standard histopathological tests.

The light microscopy showed half of the glomeruli showing mild hypercellularity with an increase in lymphocytes, there was no glomerulopathy. The interstitium was oedematous and contained a patchy infiltrate of lymphocytes and a few plasma cells and rare eosinophils. The corticomедullary junction showed a non-specific infiltrate of chronic inflammatory cells. There was peritubular capillaritis, with congestion and 2-6 luminal lymphocytes. Immunohistochemistry was negative for C4d and SV40. No glomeruli were present in the sample for electron microscopy but 10 peritubular capillaries were examined and were within normal limits. The Banff score was g1; t2; i0; ah0; mm0; cg0; ct1; ci1; cv3; ptc0; C4d0. The pathologist signed out the biopsy as ‘borderline changes suspicious for T-cell mediated rejection and acute tubular injury’.

The molecular microscope summary result was ‘Severe T Cell Mediated Rejection (TCMR) with possible Antibody Mediated Rejection (ABMR), moderate inflammation, Acute Kidney Injury (AKI) and atrophy fibrosis. This phenotype is often associated with under immunosuppression or non-adherence’. The principle component analysis is shown in Figure 1a.

After multi-disciplinary consideration and taking in to account traditional histopathology findings suspicious for TCMR, graft dysfunction and a convincing MMDx report for TCMR the patient was offered treatment with enhanced immunosuppression. In view of the patient’s recent history of PTLD it was proposed to treat with three pulses of intravenous methylprednisolone 500 mg, followed by high dose oral prednisolone and an increase in the tacrolimus dose to achieve a tacrolimus level of approximately 6 ng/mL to 8 ng/mL. Because of the presence of an anti-DP antibody and ‘Mild’ ABMR score by MMDx the patient’s donor was re-tissue typed to demonstrate that the anti-DP antibody was not donor specific. Despite high dose corticosteroids the serum creatinine did not improve (was static at 140 µmol/L [1.58 mg/dL]) and so a repeat biopsy was performed 10 weeks after the first biopsy. The light microscopy of the repeat biopsy showed 10% tubular atrophy and interstitial fibrosis with minimal interstitial inflammation. Immunohistochemistry was negative other than non-specific positivity for IgM and C1q. The Banff score was g0; t0; i0; ah0; mm0; cg0; ct1; ci1; cv3; ptc0; c4d0. The histopathologist concluded that there was no evidence of either rejection or PTLD in the allograft.

The repeat MMDx summary diagnosis was ‘No active rejection.
No active ABMR but cannot exclude late stage inactive ABMR. No TCMR. Mild AKI and atrophy-fibrosis with minimal inflammation. The mean of the two TCMR gene classifiers was ‘normal’, having been ‘severe’ previously. The visual depiction of the current sample compared to the reference set demonstrated that the biopsy had moved from a clear rejection phenotype into the no rejection zone (Figure 1b).

**Case 2**

A 68 year old female with ESRF due to APKD received a 1-1-1 ABDR mismatched kidney from a Donor after Circulatory Death (DCD). She experienced early Banff 1b TCMR in July 2012 after which allograft function was stable on maintenance immunosuppression with tacrolimus (level 5 ng/mL), azathioprine 75 mg daily and prednisolone 5 mg daily. At 6 years after transplant she was noted to have a creeping creatinine, from 100 µmol/L to 154 µmol/L (1.13 mg/dL to 1.74 mg/dL), there was no proteinuria, and the ultrasound scan was normal. There were no detectable circulating donor specific antibodies.

Consequently a kidney transplant biopsy was performed. As previously in addition to traditional histopathology a small core of cortex was sent for analysis by MMDx.

**Discussion**

These two cases demonstrate, in clinical practice, how the molecular microscope can be used to aid transplant diagnostics and guide clinical management. In the first case the MMDx report was unambiguous in diagnosing TCMR whilst the traditional histopathology was reported as ‘borderline changes’, which is an unsatisfying, ambiguous, diagnosis. This clear TCMR signal from MMDx gave the clinicians confidence to robustly treat TCMR. In the second case the pathologist diagnosed Banff 1a TCMR but the MMDx did not show rejection. The repeat biopsy did not show rejection by either traditional histopathology or MMDx and when the original
biopsy was re-reviewed by a second pathologist, blinded to the original report, it was also reported as ‘no rejection’. Relying on MMDx in the second case could have avoided unnecessary corticosteroid exposure.

The possible reasons for the discrepancy between histopathology and MMDx are clear. In the first case rejection-related changes in RNA expression will occur before tissue injury is apparent or severe. In the second case acute rejection is difficult to determine accurately in already damaged parenchyma.

Despite following the Banff schema for the classification of kidney transplant biopsies traditional histopathology involves a human factor and is, by definition, ‘interpretive’. The Banff classification is a consensus opinion of leading histopathologists. Inter-observer disagreement between histopathologists is well recognized whereas MMDx is a reproducible measure of RNA expression at the time of the biopsy. MMDx is based on analysis of gene transcripts and therefore offers an insight into the mechanisms of graft dysfunction and offers the opportunity, as in our first case, to have visual proof that treatment has altered the molecular signal from ‘abnormal’ to ‘normal’ i.e. treatment has worked at the level of gene expression. These features of MMDx make it attractive to classify alloimmune graft injury e.g. on entry to a clinical trial of treatment for rejection and also to document response, or lack thereof, to therapy.

There are disadvantages to MMDx, firstly in terms of practicality: Because the test analyses RNA transcripts in allograft biopsy it is necessary to preserve a small segment of biopsy core in RNA later at the time of the biopsy. It is not otherwise possible to retrospectively analyze the biopsy with MMDx. Furthermore at the present time MMDx is only commercially available in one laboratory, the Kashi Laboratory in Portland, OR, USA which results in some additional processing time whilst samples are transported from the clinic to the laboratory. Despite this results are returned in a clinically relevant timeframe. Secondly MMDx cannot altogether replace traditional histopathology, in part at least, because MMDx cannot exclude primary glomerular diseases, such as recurrent IgA nephropathy.

Both cases presented here corroborate claims made by Madill-Thomsen et al. [8] that ‘where histopathology is ambiguous MMDx is usually unambiguous’. The molecular microscope has the potential to improve the accuracy of diagnosis of graft dysfunction and therefore graft and patient outcomes.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References