Abstract book

How much Risk can You Take (and what to do If it all Goes Pear Shaped)
Welcome message

Welcome to the University of Warwick for our third International Transplantation meeting. We do hope you will find the programme useful. In the first two meetings we looked at HLA antibody production in the context of B cells and plasma cells. This time we are focussing on the molecular basis of antibody-antigen interactions, and we think you will find the Friday afternoon session looking at antibody interactions in a way other meetings have yet to do in the context of transplantation. The treatment of antibody mediated rejection is moving fast and we have trans-continenatal updates on best practice. We have clinical presentations on risky areas of transplantation, research presentations, debates – in all we hope you will enjoy, learn, network and contribute. We thank you for attending the meeting. Finally we would like to say an especial thank you to our sponsors for allowing us to hold an affordable meeting in luxurious surroundings.

Prof Robert Higgins, Prof David Briggs, Dr Nithya Krishnan, Dr Daniel Mitchell, Dr David Lowe, Dr Sunil Daga and Dr Natasha Khovonova

Educational Aims

By the end of the meeting it is expected that delegates will:

1. Understand the results of current therapies for antibody mediated rejection
2. Understand the principles of antibody-antigen interactions and how these might be disrupted in the future
3. Be aware of current practice in high risk areas of renal transplantation, in relation to donor and recipient obesity
4. Have an understanding of the outcomes and limitations of current methods for measuring HLA and ABO antibody levels, and how these results might be related to clinical outcomes
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Program

Day 1: Friday 31st October 2014

0900 -0925  Registration and Coffee

0925-0930  Welcome - Prof Robert Higgins & Dr Dan Mitchell

Morning session:

0930-0950  Antibody mediated rejection, a UK perspective
Prof Rob Higgins, Coventry, UK

0950-1010  Antibody mediated rejection; New insights into diagnosis, activity
and pathophysiology
Dr Alexandre Loupy, Paris, France

1030-1110  Antibody mediated rejection; a USA perspective
Dr Babak Orandi, Johns Hopkins Hospital, Baltimore, USA

1110 – 1130  Coffee

1130-1200  Successful treatment of antibody mediated rejection in ABO
incompatible transplantation
Dr Rachael Coates, Newcastle, UK

1200-1300  Oral abstract presentations

1. Transplant strategies for highly sensitised patients
James Kelleher, Derek O’Neill, David Keegan, Patricia Mullaney, Catriona Leslie, Julie
Purcell, Jane Kelly-Rogers, Joseph Kelly, Susan Jennings, Catherine Taylor, Grainne
Harmon, Deirdre Keane, Geraldine Donnelly, Claire Lenahan, Natasha Caulfield, Micheal
Doughty, Michelle Gardner, Caroline Coleman, Jimmy Gooi, Khairin Khalib, Mary Keogan.
National Histocompatibility and Immunogenetics Service for Solid Organ Transplantation
(NHISSOT), Beaumont Hospital Beaumont, Dublin 9, Ireland.

2. Irish Experience of Participation in UK Paired Kidney Exchange (PKE) Scheme
Connaughton D.M, Canney M., O’Regan J., Williams Y., Cunningham P., Hart P., O’Neill D.,
Department of Nephrology and Transplantation, Beaumont Hospital, Dublin 9.

3. Integrating Desensitisation with the National Kidney Sharing Scheme
Siân Griffin¹, Emma Burrows², Sandra Lloyd², Rhian Cooke¹, Ann Marsden¹, Argiris
Asderakis¹, Tracey Rees²
¹Cardiff Transplant Unit and ²Welsh Transplantation and Immunogenetics Laboratory
4. Decision trees in renal transplantation: prediction of acute antibody mediated rejection in the early post-transplant period

Torgyn Shaikhina1, Sunil Daga2,4, Nithya Krishnan4, Dave Lowe3, Daniel Mitchell2, David Briggs2, Robert Higgins4 & Natasha Khovanova1
1School of Engineering, University of Warwick; 2Warwick Medical School, 3NHSBT Birmingham, 4UHCW NHS Trust, Coventry

1300-1400 Lunch

Afternoon session:

1400-1430 Complement binding and the cytotoxic crossmatch – unpicking a 50 year old test with 21st century methods
Dr David Lowe, Liverpool, UK

1430-1500 Towards predicting HLA alloantigen immunogenicity: application of three-dimensional structural and computational modelling
Dr Dermot Fallon, Cambridge, UK

1500-1530 Characterisation of binding of antibody to HLA-antigen using biosensor assay: tool to define pathogenicity of antibodies
Dr Sunil Daga, Coventry, UK

1530-1600 Coffee

1600-1630 Innovations to disrupt pathogenic antibody-antigen interactions
Prof Richard Pleass, Liverpool, UK

1630-1645 Day closes
DAY - 2 Saturday 1st November 2014:

Parallel clinical session

0900 -0925 Registration and Coffee

0925-0930 Welcome - Dr Nithya Krishnan & Prof Robert Higgins

Clinical session 1:

0930-1015 Obese patients - is it right to deny transplantation?
Dr Nithya Krishnan, Coventry, UK

1015-1100 Donor BMI – How high can we go?
Dr. Babak Orandi, John Hopkins, Baltimore, USA

1100-1130 Coffee

Joint sessions

11:30-1215 Tolerance – is this really the way forward or can it only be seen in culture plates?
Prof. Kathryn Wood, Oxford, UK

Clinical session 3:

1215-1300 Risks of donor transmitted malignancies
Prof James Neuberger, Birmingham, UK

1300- 1400 Lunch

Clinical session 4:

1400-1445 Cardio vascular assessments in recipient and donor work up
Dr. Paul Harden, Oxford, UK
Clinical session 3:

1445-1530  Short oral abstracts

1. Exercise pressor response in an end-stage renal failure patient with chronic refractory hypotension: before and following kidney transplantation
   Alice Rogan¹, Stephen Ting¹,³, Gordon McGregor¹, Charles Weston², Nithya Krishnan¹, Robert Higgins¹, Daniel Zehnder¹,³.
   ¹Department of Renal Medicine and Transplantation, University Hospital Coventry and Warwickshire NHS Trust, Coventry, UK; ²Department of Nephrology, Dorset County Hospital NHS Foundation Trust, Dorchester, UK; ³Division of Metabolic and Vascular Health, University of Warwick, Coventry, UK.

2. An audit of post-transplant magnesium levels
   Ines Held*, Karen Pickles**, Robert Higgins **, Peter Rutherford**
   * Wrexham Maelor Hospital Croesnewydd Road, Wrexham LL137TD, ** UHCW, Clifford Bridge Road Coventry CV2 2DX, ** Glyndwr University, Mold Road Wrexham LL112AW

3. Impact of Type Tacrolimus Assay on Therapeutic Drug Monitoring: Need for Standardization
   Mansumeet Singh, Siddig Anwar, Rowena Delos Santos, Daniel C Brennan. Washington University School of Medicine, St Louis, USA

4. Varied presentations of post renal transplant malignancies.
   V. Ravi, K. Gopalakrishnan, R. Higgins & N. Krishnan. Department of Renal Medicine and Transplantation, University Hospital Coventry and Warwickshire NHS Trust, Coventry, UK

5. Is myocardial perfusion scan an effective screening tool in pre transplant work up?
   V. Ravi, J. Cullis, N. Williams, N. Aldridge, Y. Myers, N. West, R. Higgins and N. Krishnan. Department of Renal Medicine and Transplantation, University Hospital Coventry and Warwickshire NHS Trust, Coventry, UK

1530-1600  Coffee

Joint sessions

1600 – 1700  Debate- 'Renal Transplant Allocation should take more account of Transplant Outcomes, especially in relation to allocating kidneys to elderly recipients'
   Chair- Prof. James Neuberger
   For - Prof Robert Higgins & Against - Prof Anthony Warrens UK

1700 – 1730  Day closes
DAY - 2 Saturday 1st November 2014:

Parallel H&I/ Immunology session

0925-0930  Welcome: Prof David Briggs & Dr David Lowe

H&I session 1:

0930-1000  The art of defining HLA-specific antibodies
            Prof Derek Middleton, Liverpool, UK

10:00–11:00 Oral Abstracts

1. Comparison of the Complement dependent cytotoxicity assay with the Single antigen bead assay for the identification of HLA specific antibodies
   Maria Irvine1,2, James Jones2, Derek Middleton2, Dave Lowe2
   1School of Pharmacy and Biomolecular science, Liverpool John Moores University
   2Transplant Immunology, Royal Liverpool and Broadgreen University Hospital Trust

2. Presence and levels of non-complement fixing IgG4 subclass associates with early graft rejection and decreased allograft survival times in antibody incompatible transplantation
   Natasha Khovanova1, Dave Lowe3, Sunil Daga2,4, Torgyn Shaikhina1, Nithya Krishnan4, Daniel Mitchell2, Daniel Zehnder2,4, David Briggs3 & Robert Higgins4
   1School of Engineering, University of Warwick; 2Warwick Medical School, 3NHSBT Birmingham, 4UHCW NHS Trust, Coventry

3. The correlation of IgG subclasses with clinical outcomes for ABO incompatible renal transplants
   MBraitch1, A.Bentall2, S Daga2, D.Briggs, S.Ball
   1Renal Research group, University of Birmingham, Queen Elizabeth Hospital B15 2TY;
   2NHSBT, Vincent Drive, Edgbaston, Birmingham, B15 2SG

1100-1130  Coffee

Joint sessions

11:30-1215  Tolerance – is this really the way forward or can it only be seen in culture plates?
            Prof. Kathryn Wood, Oxford, UK
H&I session 2:

1215-1300 sessions 2 - Oral Abstracts

   
   James Jones¹, Dave Lowe¹, Maria Irivine¹,² and Derek Middleton¹
   
   ¹Transplant Immunology, Royal Liverpool University Hospital Trust; ²School of Pharmacy and Biomolecular Science, Liverpool John Moores University

2. Mathematical models for estimating binding kinetics of antibody–antigen interactions
   
   H Moyse¹, S Daga²,³, D Lowe³, N Krishnan⁴, D Briggs³, R Higgins²,⁴, D Zehnder²,⁴, D Mitchell² and N Evans¹
   
   ¹School of Engineering, University of Warwick; ²Warwick Medical School, ³NHSBT Birmingham, ⁴UHCW NHS Trust, Coventry

1300- 1400 Lunch

H&I session 3:

1400-1500 Debate – ‘CDC: 50 years is enough! There must be something better’

Chair/ Referee: Derek Middleton

For Prof Robert Vaughan and Against Prof David Briggs

1500-1530 Case studies – How high is high antibody

1530-1600 Coffee

Joint sessions

1600 – 1700 Debate- 'Renal Transplant Allocation should take more account of Transplant Outcomes, especially in relation to allocating kidneys to elderly recipients'

Chair- Prof. James Neuberger

For - Prof Robert Higgins & Against - Prof Anthony Warrens UK

1701 – 1730 Day closes
Abstracts - Day 1

1. Transplant strategies for Highly sensitised patients

Patients with a cRF of 100% have limited access to transplantation, and may require transplantation across circulating and historic antibodies. We have previously shown that single-technique antibodies (STAs) detectable by only one Luminex single-antigen assay are not clinically relevant. We undertook a longitudinal review of assigned antibodies for highly-sensitised patients reviewing antibody strength, sensitisation source, method(s) of detection and period of positivity.

We reviewed the outcome of antibody reviews for patients with a Pgen of 100%, in whom historic antibodies and STAs were no longer considered unacceptable. We reviewed all patients transplanted across donor-specific STAs. STAs are routinely crossed in clinically urgent patients, paediatric patients and in living donor transplantation.

Seven of 17 (41%) patients have been transplanted following review. All were transplanted across historic antibodies. Three patients had positive flow crossmatches, however crossmatches were deemed to be non-HLA related.

Twenty-one patients have been transplanted across circulating antibodies (MFI’s 513-5709), without antibody removal. Fourteen patients had positive flow crossmatches (two T and B, one T cell and 11 B cell).

Mean follow-up of these 28 transplanted patients was 32 months (range 10-57 months). Graft survival is 93% (one graft lost to thrombosis, one death with a functioning graft). There has been no AMR, 2 biopsy-proven cellular rejections, and one steroid boost for presumed rejection. 27/28 have a creatinine <200mmol/l, with median creatinine of 109 (range72-325mmol/l).

Long-term outcome may not be as favourable as antibody compatible transplantation, but this initiative facilitates access to transplantation for these patients.
2. **Irish Experience of Participation in UK Paired Kidney Exchange (PKE) Scheme**

Presenting authors affiliation: Department of Nephrology and Transplantation, Beaumont Hospital, Dublin 9. dervlaconnaughton@gmail.com

**Background:** In Ireland patients with a living donor, to whom they are deemed immunologically incompatible, are referred to the United Kingdom for enrolment into the PKE programme. This allows the incompatible donor-recipient pair to be matched with another incompatible pair in order to proceed with live kidney transplantation. Here we report the Irish experience.

**Methods:** Retrospective chart review of all patients enrolled in the PKE programme from 2010.

**Results:** In total 35 donor-recipient pairs have been listed in the PKE programme, with 18 pairs currently active on the list. The majority of patients referred are highly sensitised (74.2% (n=36) PGen >80%) and approximately 1/3 of recipients are blood group O. Three (8.5%) donor-recipients pairs have undergone successful PKE transplantation all of whom now have functioning grafts; one pair is scheduled for surgery shortly. Six patients (17%) proceeded to direct living donor transplantation of whom 4 requiring desensitization prior to transplantation. Three recipients received a deceased donor kidney transplant from the Irish pool. The average number of runs in the PKE programme, with 1 run occurring each quarter, is 2 for successful pairs. However the average numbers of for all patients listed is 5.25 runs (range 1 -28 runs).

**Conclusion:** PKE remains an option for incompatible donor-recipient pairs which should be explored prior to desensitization and can offer patients who are highly sensitized the option of a living donor transplant. Current success rates for Irish patients are approximately 11% with a trend toward lower success rates in the highly sensitized group.
3. Integrating Desensitisation with the National Kidney Sharing Scheme

**Siân Griffin¹**, Emma Burrows², Sandra Lloyd², Rhian Cooke¹, Ann Marsden¹, Argiris Asderakis¹, Tracey Rees²

¹Cardiff Transplant Unit and ²Welsh Transplantation and Immunogenetics Laboratory

**Introduction:** The National Living Donor Kidney Sharing Scheme (NLDKSS) has increased the opportunity for HLA sensitised recipients to receive a compatible transplant. However, for those with a cRF > 95%, the chance of finding a match is low.

**Methods:** The unacceptable HLA antigen profile of 5 sensitised patients entered in the NLDKSS was modified to remove non-cytotoxic antibodies with an MFI <5000. This profile was used in anticipation of achieving a cytotoxic negative, flow cytometry positive cross match that would be amenable to desensitisation. The desensitisation regimen comprised Rituximab 4 weeks prior to transplantation, double filtration plasmapheresis (DFPP), induction with Alemtuzumab and triple oral immunosuppression.

**Results:** Potential matches leading to transplantation were identified for all 5 recipients in runs 1 (n=1), 2 (n=3) and 3 (n=1). Four recipients had positive cytotoxic cross matches with their original donors. Cross matches with NLDKSS donors were all cytotoxic negative, four were flow cytometry positive. Median cRF prior to de-listing antibodies was 99% (range 72 – 100%), and after delisting 87% (range 64 – 96%). All grafts functioned immediately. Two recipients received DFPP post-transplant in response to a rise in donor specific antibodies. The recipients are now 3 – 27 months post-transplant. One patient had two episodes of borderline cellular rejection and none had antibody mediated rejection. The mean eGFR at last follow up was 69±17 ml/minute, and no recipients have proteinuria.

**Conclusion:** Transplantation of selected highly sensitised recipients can be facilitated using a combination of desensitisation and the NLDKSS.
4. Decision trees in renal transplantation: prediction of acute antibody mediated rejection in the early post-transplant period

Torgyn Shalkhina, Sunil Daga, Nithya Krishnan, Dave Lowe, Daniel Mitchell, David Briggs, Robert Higgins & Natasha Khovanova

1School of Engineering, University of Warwick; 2Warwick Medical School; 3NHSBT Birmingham; 4UHCW NHS Trust, Coventry

This research presents a novel application of classification Decision Trees (DTs) for prediction of acute antibody mediated kidney transplant rejection. In the dynamic field of renal transplantation the vast potential DTs is not sufficiently explored due to costly experiments, underlying complexity and uncertainty over causality in the data. We demonstrate that the DT approach can successfully identify the optimal hierarchy of principal parameters associated with the early graft rejection for risk assessment preceding transplantation.

The available clinical dataset featured 15 potential predictor variables, including pre-transplant DSA IgG subclass levels across 46 rejector (R) and 34 non-rejector (NR) observations. 60 samples were used for DT training and the remaining 20 were reserved for testing how well the DT classifier generalises on new data. In order to compensate for high volatility in performance due to small number of training samples, 600 separate DTs were investigated. The best performing model achieved 86.7% accuracy during training and correctly classified 85% of test cases. This DT classified the data into the R/NR groups based on 6 variables (top to bottom in Figure 1): the highest MFI DSA level (root), total IgG4 MFI, HLA mismatch number, total IgG2 MFI, delayed graft function and the total IgG1 MFI. The node splits provide an indication as to what specific levels of the DSA antibodies pre-transplant are statistically associated with each of the R/NR groups. For instance, the DT identified that patients with the highest MFI DSA levels below 834 belong to a NR group, while those with the total IgG4 levels ≥ 36.5 have a high likelihood of early transplant rejection.

Overall, within the limited input dataset, the DT model provides accurate predictions for kidney transplant rejections, whilst simultaneously estimating the highest risk factors and the specific antibody subclass levels associated with the increased risk of rejection in the early post-transplant period.

![DT structure](image)

Figure 1 - DT structure
Chronic refractory hypotension is a rare but significant mortality risk in renal failure patients. Such aberrant physiology usually deems patient unfit for renal transplant surgery. Exercise stimulates the mechano-chemoreceptors in the skeletal muscle thereby modulating the sympathetic effects on blood pressure (BP) regulation; this is known as the exercise pressor effect. The haemodynamic response to dynamic exercise in such patients has not been previously investigated. We present a case with severe chronic hypotension who underwent exercise testing before and after renal transplantation, with marked differences in BP response to exercise.

A 40-year-old female haemodialysis-dependent patient with a two year history of refractory hypotension (≤ 80/50 mmHg) (Figure) underwent haemodynamic evaluation during cardiopulmonary exercise testing. BP normalized during unloaded pedaling but was exaggerated at maximal workload whereby BP rose from 82/50 mmHg to a peak of 201/120 mmHg. She underwent an antibody-incompatible renal transplantation. Eight weeks following transplant, resting BP was normal and a physiological exercise-haemodynamic response was observed (Figure).

Our results suggest that there may be a therapeutic role of dynamic exercise in ESRD to correct intradialytic hypotension, thus allowing greater tolerance to fluid shift. The case study adds to the evidence that sympathetic dysfunction is reversible with renal transplant. Furthermore chronic hypotension with preserved cardiovascular reserve should not preclude these patients from transplant surgery.
2. An audit of post-transplant magnesium levels

Ines Held*, Karen Pickles**, Robert Higgins **, Peter Rutherford***

* Wrexham Maelor Hospital Croesnewydd Road, Wrexham LL137TD, ** UHCW, Clifford Bridge Road Coventry CV2 2DX, *** Glyndwr University, Mold Road Wrexham LL112AW

Background - Low magnesium levels have been associated with poor cardiovascular outcomes mediated via hypertension and endothelial dysfunction. Ischaemic graft damage and drugs used in immunosuppressive protocols may predispose renal transplant patients to magnesium wasting.

Methods – Retrospective audit examining magnesium levels pre and post (approximately one month) transplantation at University Hospitals Coventry and Warwickshire Hospital. Data from 208 patients, over 35 months, were examined - 112 live related, 96 cadaveric or non-heart beating donors. Lowest level, procedure data and number of magnesium estimations per patient were collected.

Results – Magnesium levels fell significantly following transplantation (pre vs post, * P=0.002)

<table>
<thead>
<tr>
<th></th>
<th>Pre (mean±SD)</th>
<th>Post</th>
<th>Lowest</th>
</tr>
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<tbody>
<tr>
<td>Serum magnesium</td>
<td>0.905±0.184</td>
<td>0.703±0.149*</td>
<td>0.616±0.103</td>
</tr>
</tbody>
</table>

Time between transplant and lowest value was variable median 14 days (range 0 to 1169 days). Number of tests performed post transplantation was variable – median 12 (range 5 to 64 tests). There were significant correlations between cold ischaemic time (CIT) and post ($r^2 = 0.189$, p=0.008) and lowest levels ($r^2 = 0.330$, p<0.0001).

Discussion – Magnesium levels fell post transplant with a variable time to lowest level. The number of magnesium levels performed varied suggesting the need for protocol driven care. CIT is important but other factors should be closely examined.
3. **Impact of Type Tacrolimus Assay on Therapeutic Drug Monitoring: Need for Standardization**

Mansumeet Singh, Siddig Anwar, Rowena Delos Santos, Daniel C Brennan

Washington University School of Medicine, St Louis, USA

**Background:** Multiple assays are available for measuring tacrolimus (FK) levels and these are not currently standardized. A designated laboratory transitioned their FK assay from Enzyme Multiplied Immunoassay Technique (EMIT) to Liquid Chromatography with Tandem Mass Spectrometry (LC-MSMS) during April and May 2013.

**Methods:** We analyzed the data on 393 patients transplanted between Jan 1993 and Dec 2012 from this designated laboratory who were on a stable dose of FK. The values of FK levels between January 2013 and August 2013 (3 months before and after the assay change) were also analyzed for these patients for comparison. Continuous variables are described as means with SD. The proportion of patients requiring dose change was described as a percentage. Paired T-tests were used to compare the mean FK level before and after the assay change.

**Results:** The table shows the results of the paired t-tests. There was a significant difference in mean FK levels before and after the change in assay. After adjustment in FK dose, there was no significant difference in the mean FK levels. (Table-1)

**Conclusion:** This study highlights the importance of the type Tacrolimus assay on the FK levels and urgent need for standardization. The implementation of liquid chromatography with tandem mass spectrometry (LC-MSMS) technique as a standard could help standardize the reporting of FK levels. There is an urgent need to develop best practice guidelines for LC-MSMS methods for its use for routine FK measurement. This will help harmonize results across diagnostic laboratories.

**Table: 1**

<table>
<thead>
<tr>
<th>Pairs</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>1) Month 1</td>
<td>5.93</td>
<td>1.71</td>
<td>0.075</td>
</tr>
<tr>
<td>1) Month 3</td>
<td>5.65</td>
<td>1.86</td>
<td></td>
</tr>
<tr>
<td>2) Month 1</td>
<td>5.95</td>
<td>2.14</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2) Month 4</td>
<td>5.33</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>3) Month 1</td>
<td>5.90</td>
<td>2.16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>3) Month 5</td>
<td>3.76</td>
<td>1.57</td>
<td></td>
</tr>
<tr>
<td>4) Month 5</td>
<td>3.77</td>
<td>1.76</td>
<td>0.297</td>
</tr>
<tr>
<td>4) Month 7</td>
<td>3.63</td>
<td>1.58</td>
<td></td>
</tr>
<tr>
<td>5) Month 5</td>
<td>3.86</td>
<td>1.76</td>
<td>0.154</td>
</tr>
<tr>
<td>6) Month 8</td>
<td>3.61</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>53.95</td>
<td>14.92</td>
<td></td>
</tr>
<tr>
<td>Required dose change</td>
<td>60 (15.3%)</td>
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</tbody>
</table>
4. Varied presentations of post renal transplant malignancies.

V. Ravi, K. Gopalakrishnan, R. Higgins & N. Krishnan
Department of Renal Medicine and Transplantation, University Hospital Coventry and Warwickshire NHS Trust, Coventry, UK

Introduction: Malignancy represents a major burden post transplantation. We report 3 cases with different presentations of post-transplant malignancies.

Case Descriptions: Case 1 is a 46-year-old patient who reached end stage renal failure due to hyperoxalosis, secondary to Crohn’s disease. Transplant nephrectomy was performed at the time of second kidney transplant. The histology showed multiple lesions of renal cell carcinoma. The patient presented with no symptoms.

Case 2 is of a young male with renal failure secondary to congenital bladder exstrophy leading to obstructive uropathy. He had bilateral nephrectomy and an ileal conduit and a cadaveric transplant when he was 10 years of age. 22 years later, the patient had a HLA incompatible from his father. Four years later he presented with haematuria. MRI did not demonstrate any abnormality. Ten months after he presented with spiking fever and pain in the left iliac fossa. A CT showed chronic obstruction of his first transplant kidney and had transplant nephrectomy which showed adenocarcinoma arising from the ileal conduit.

Case 3. A 60 year old female diagnosed with IgA nephropathy received renal transplant from her son. She complained of generalized back pain but more pronounced on her right. Initial CT scans did not show much, a repeat CT 24 months later revealed a 2.5 cm right renal carcinoma in the upper pole of right kidney. She subsequently underwent a right nephrectomy and histology confirmed renal cell carcinoma with no metastasis. Six months later, she presented with right hip pain and was found to have extensive metastasis.

Though yearly US has been suggested as a screening tool for malignancy post-transplant, the above cases demonstrate that imaging does not always detect early stages of malignancy. Perhaps novel serum marker assays for the early detection of malignant tumors would be the way forward.
5. Is myocardial perfusion scan an effective screening tool in pre transplant work up?

V. Ravi, J. Cullis, N. Williams, N. Aldridge, Y. Myers, N. West, R. Higgins and N. Krishnan. Department of Renal Medicine and Transplantation, University Hospital Coventry and Warwickshire NHS Trust, Coventry, UK

Background: There is no definitive consensus for pre-transplant cardiovascular screening at present. Our current protocol includes echocardiogram (ECHO) and cardiopulmonary testing (CPEX) for all patients; for patients 50 years or over, previous transplants, diabetes or on dialysis for longer than 2 years, myocardial perfusion imaging (MPS) is included as an additional test.

Aim: We looked to see if MPS is an effective screening tool for diagnosing coronary artery disease in pre transplant work up.

Methods: All patients in our centre, who underwent MPI, CPEX and ECHO between January 2012 to September 2014 were investigated. There were 103 patients, aged between 30 to 80 years, 67 (65%) were males. There were 30 diabetics in total, mostly comprising of male patients (83%).

Results: The table below shows the MPS reversibility score, CPEX anaerobic threshold (AT), ECHO left ventricular ejection fraction (EF) and history of ischaemia heart disease (IHD) for the patient group.

<table>
<thead>
<tr>
<th>MPS</th>
<th>No</th>
<th>AT &lt;7</th>
<th>AT = 7-11</th>
<th>AT &gt;11</th>
<th>EF &lt;30%</th>
<th>EF = 30-50%</th>
<th>EF &gt;50</th>
<th>History of IHD</th>
<th>Post MPS MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ischemia</td>
<td>94</td>
<td>4</td>
<td>52</td>
<td>38</td>
<td>1</td>
<td>6</td>
<td>87</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Mild ischemia (&lt;10)</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Severe ischemia (&gt;10)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
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</table>

Conclusions:

Our study, showed that the MPS identified significant ischaemia in a single patient with known IHD. The study population was small but there was no evidence of correlation between the various investigations. Given the radiation exposure of the MPS investigation (typically > 8mSv) and the cost, should MPS investigations be considered only on patients with known IHD or patients with symptoms of IHD accompanying significant risk factors? Or is there a role for TropT, BNP and other cardiac markers?
Abstracts Day 2 H&I

Session 1:

1. **Comparison of the Complement dependent cytotoxicity assay with the Single antigen bead assay for the identification of HLA specific antibodies**

   Maria Irvine¹,², James Jones², Derek Middleton² Dave Lowe²

   ¹School of Pharmacy and Biomolecular science, Liverpool John Moores University
   ²Transplant Immunology, Royal Liverpool and Broadgreen University Hospital Trust

**Aim:** To compare the CDC assay and SAB assay for the detection of HLA specific antibodies, focusing on the highly immunogenic HLA-A2 or HLA-B7 antigens.

**Method:** Patient sera with an HLA-A2 or HLA-B7 specific antibody were selected for CDC analysis. The sera was dispensed into microcytotoxicity trays and tested against 65 different HLA cell types. The same patients were also analysed by SAB.

**Results:** 10 of 67 patients only reacted with a mono-specific antigen by CDC, compared to the SAB were the average number of specificities was 31 (range 8-58). In 5 of 10 patients, the equivalent MFI of the monospecific antigen determined by CDC was less than 5,000 and in 3/5 it was less than 1,300. One patient was investigated by epitope analysis of SAB which identified poor correlation between MFI and epitope number. When the sera was diluted a better correlation between MFI and multiple epitopes was revealed.

**Conclusion:** The data demonstrates that the CDC assay provides a level of interaction between antibodies to multiple epitopes that cannot be revealed by SAB alone. The CDC identified antigens that would not always be above the routine MFI threshold. In comparison SAB epitope analysis helped to define the number of possible reactive epitopes. Serum dilution allowed a better correlation between MFI and reactive epitope number.
2. Presence and levels of non-complement fixing IgG4 subclass associates with early graft rejection and decreased allograft survival times in antibody incompatible transplantation

Natasha Khovanova¹, Dave Lowe³, Sunil Daga², ⁴, Torgyn Shaikhina¹, Nithya Krishnan⁴, Daniel Mitchell², Daniel Zehnder², ⁴, David Briggs³ & Robert Higgins⁴
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It is known that donor specific antibodies (DSA) represent a risk factor for early transplant rejection and influence allograft survival times. The aim of this research was to investigate the role of all DSA IgG subclasses (1-4) in the immune response in order to identify any potentially damaging antibodies and their influence on short and long postoperative outcome.

77 transplanted samples were available for short-term outcome analysis comprising 43 cases experiencing rejection (R) episodes within the first 30 days after transplantation and 34 cases of non-rejectors (NR). IgG (1-4) DSA levels were determined for all pre-transplant, peak and 30th day post-transplant samples. We also divided the samples into 2 groups according to long-term outcome: there were 14 samples with failed transplants and 57 with functioning allograft.

We have demonstrated that IgG4 subclass was predictive of acute antibody mediated rejection (p=0.003) in the early post-transplant period. The multiple binary regression analysis has shown that the occurrence of the early rejection was due to 3 factors: total IgG4 MFI levels, the highest MFI DSA levels and the total number of HLA mismatches. For each additional mismatch there was 3.2 times increase in the odds of the transplant being rejected; 13% increase in the odds of the rejection for each 1000 units increase in MFI highest IgG; and per each 1000-unit increase in total IgG4 levels there was 30% increase in the odds of the transplant being rejected.

Long term graft survival times were also affected by the increased presence of the IgG4 subclass in pre-transplant samples: Kaplan-Meier survival analysis showed that death censored graft survival was significantly worse in cases with pre-transplant IgG4 positive compared to cases with negative IgG4 (p = 0.004). This conclusion is supported by the multivariate Cox proportional hazard method where 2 significant (p<0.05) factors (MFI highest IgG and presence of total IgG4 in pre-transplant samples) showed the hazard ratios of 161.218 and 5.945, respectively.

These results highlight a clear link between the presence of IgG4 subclass in serum before transplantation and unwanted postoperative outcomes such as acute antibody mediated rejection and long term graft failure. Therefore, IgG4 can be additional biomarker that could be used to risk stratify kidney transplant recipients.
3. The correlation of IgG subclasses with clinical outcomes for ABO incompatible renal transplants

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Introduction

Complement activation occurs in antibody binding in transplant recipients with anti-donor antibodies. It is associated with antibody-mediated rejection but is also present in ABO incompatible kidney transplantation without adverse clinical outcomes. Immunoglobulin subclasses (IgG 1, 2, 3 and 4) differ in their ability to activate the complement cascade and trigger rejection. Whilst higher anti-A/B titres have been associated with poor outcomes (including graft loss and antibody-mediated rejection), the majority of patients with higher titres have good outcomes. In order to risk assess patients, we investigate the IgG subclass profile of patient before desentisation in order to describe the distribution and correlate these with clinical events including graft loss and antibody-mediated rejection. We also aimed to correlate the IgG subclass data with IgG and IgM haemagglutination data.

Method

A flow cytometric assay was used to quantify the IgG subclasses, in a cohort of 100 ABO blood group incompatible kidney transplant patients from the ABOUT-K study. Fixed red blood cells were incubated with plasma, secondary antibodies were added. Cells were washed with PBS, centrifuged and read on a FACS canto flow cytometer. Plasma samples were analysed during pre-induction, pre-extracorporeal antibody removal therapy (EART) and pre-transplant.

Results

The mean age of the recipients was 48.1 ± 13.6 years, 41% were female, 68% were blood group O. A one year follow up time was reached in 80% of patients. IgG 1, 3 and 4 level were below the limit of detection. (Mean Relative MFI ≤ 1.5). However IgG2 was quantified in all pretransplant samples (Mean RMFI±SD) (pre-induction, 11 ± 2.9, pre-EART, 4.3 ±0.96 and pre-txp, 3.3 ± 1.1). There was no statistical significant difference between IgG2 vs IgM and IgG from patients, who rejected, (Mann Whitney, p=0.53, p=0.68). Further to this no statistical significant differences were observed between IgG vs IgM (Mann Whitney, p=1.00) in this group of patients.

Conclusion

This is the first study which has investigated IgG subclasses in sera from pre-transplant patients and explored their association with clinical outcomes for ABO incompatible kidney transplants. We can report that IgG subclasses do not serve as a biomarker for rejection.
Session 2:


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Background

Problems with HLA-specific antibody analysis are associated with the polyclonal nature of the immune response, the extensive polymorphism of the HLA system, linkage disequilibrium and cross-reactivity.

But the incorporation of epitope data into the analysis of phenotype- (Cell/PRA) and SAB-based assays, allows these problems to be overcome and give greater resolution in antibody assignment.

However, as most epitopes are associated with multiple antigens, analysis can become slow and laborious and therefore not easy to routinely perform.

Aim

The aim of this project was to develop software-based algorithms to perform antigen and epitope analysis for cell-based and SAB-based assays. Key considerations were to provide a standardised, robust and automated analysis of assay data.

Methods

Conventional methods of antibody analysis was revisited and components of which were adjusted to give a more objective analysis. Antibody data generated from CDC and SAB assays were run through the algorithm and compared with manual interpretations. Parameters such as antigen frequency, epitope frequency and multiple epitope combinations were analysed.

Results

Automated epitope analysis of SAB allows maximum epitope counts to easily be calculated for each antigen above a given MFI threshold. For antigens below a set threshold, epitope analysis can be used to calculate an inclusion index based upon sensitivity by which to set a more appropriate MFI threshold. Applying this analysis to the cell-based assay revealed correlations between positivity and epitope copy number or the conformational arrangement between multiple epitopes.
2. Mathematical models for estimating binding kinetics of antibody–antigen interactions

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Antibody-antigen interactions are the first step in an immune response. Following these interactions, effector mechanisms are initiated resulting in pathological damage/response. Current assays are able to give limited detail about the specificity of patient antibody for donor antigen, particularly in the case of Human Leucocyte Antigen (HLA).

We have developed a surface plasmon resonance assay to study interactions of HLA protein and HLA-specific antibodies as well as robust mathematical and computational techniques to estimate the affinities of HLA antibodies, ABO antibodies, antibody in polyclonal mixtures, monoclonal antibody and antibody in monoclonal mixtures.

In my talk I will give an overview of the mathematical models that are commonly available for use with surface plasmon resonance experiments, their relative strengths and weaknesses for use in determining antibody antigen affinities, and present novel models that overcome these problems, as well as comparing the fits that they give to experiments performed with refined patient antibody and monoclonal antibody.

Figure 1 shows a comparison between a pre-existing model and one of these new models and their fits to data.

A) Bivalent model

B) Bivalent effective rate constant with spatial effects (bERCs) model

Figure 1. Comparison between two models fits (black) of Wk1d12 antibody in three different concentrations, 100 nM (Blue), 50 nM (green), 25 nM (red), binding to B7 antigen; A) Bivalent model B) Bivalent effective rate constant with spatial effects (bERCs) model

Session 3 – case studies – How high is the high antibody levels
Biography of organisers / speaker-

Prof Robert Higgins, MB ChB, FRCP, MD

Prof Robert Higgins is Head of Transplantation at University Hospitals of Coventry and Warwickshire NHS Trust and Honorary Professor in the University of Warwick. He has developed the programme of intensive research and development focusing on innovative methods to remove antibody from patients, have produced the most detailed descriptions of changes in antibody levels post-transplant, and identified ways of improving the standards for diagnosing antibody-mediated rejection on biopsy. He was instrumental to the first renal department unit in the UK to have commissioner support for antibody incompatible transplants and proposed a National Registry, which is the first of its type in the world.

Prof David Briggs MSc PhD

Prof David Briggs leads the Department of Histocompatibility and Immunogenetics at NHSBT, Birmingham, which provides a regional service to solid organ and stem cell transplant units in the West Midlands. He is an Honorary Professor in the School of Cancer Sciences at the University of Birmingham, UK. Prof Briggs research interests include genetic variations within the immune system that lead to variations in clinical outcomes and the heterogeneity of the humoral response against transplantation antigens.

Dr Nithya Krishnan, MBBS, MRCP, MD

Dr Krishnan is a Consultant Transplant Nephrologist at University Hospitals of Coventry & Warwickshire NHS Trust Coventry, UK and Honorary Associate Professor at University of Warwick, UK. Her research interest is antibody incompatible kidney transplantation, cardiovascular and obesity risks in transplant recipients. She has described use of flow cytometry to measure ABO antibodies following ABO-incompatible kidney transplantation.

Dr Daniel Mitchell PhD

Dr Daniel Mitchell is an Associate Professor at the University of Warwick. Dr Mitchell's obtained PhD degree (awarded in 2000 from Imperial College). Research interests include the molecular basis for immune system interactions with their environments, especially the role of glycobiology and the complement system in pathogen recognition and cellular communication in host tissues. Recent work involves the analysis of polymer-protein and polymer-cell interactions in healthcare, drawing upon strengths in polymer chemistry at Warwick. These studies are being developed alongside clinical studies in renal transplantation at the University Hospital, Coventry.
Dr David Lowe, MSc PhD

Dr David Lowe is Clinical Scientist and Deputy Head of the Histocompatibility and Immunogenetics Laboratory at Royal Liverpool University Hospital and Honorary Senior Lecturer at University of Liverpool. He previously worked in the Histocompatibility and Immunogenetics Laboratory NHS Blood and Transplant Birmingham. Dave has 16 years’ experience relating to all areas of solid organ transplantation, stem cell transplantation and immunogenetics. Has particular interest in the study of the HLA-specific antibody response, completing a thesis entitled ‘Characterisation of HLA-Specific Antibodies’ from the University of Warwick. In addition he has published over 20 papers primarily looking at HLA-specific antibodies and their characteristics, in particular focussing on HLA antibody incompatible transplantation.

Dr Sunil Daga MBBS MRCP (Nephrology)

Dr Sunil Daga did degree in Medicine at M S University, Vadodara India in 2002. He completed training in Renal Medicine in 2011. He has worked in transplant centres at Leicester, Nottingham and Coventry. He completed CLRN mentorship in 2013. He is a Research Fellow in Kidney Transplantation (Immunology) and is a sub-investigator for number of clinical trials. He is currently completing his PhD at the University of Warwick with the research focus on binding kinetics of HLA-specific antibodies. His other research work includes development of multi-colour flow cytometry assay to measure ABO antibodies, evolution of de novo HLA-specific antibodies and the role of donor released soluble HLA proteins following kidney transplantation.

Dr Natasha Khovanova PhD

Dr Natasha Khovanova is an Associate Professor in the School of Engineering at the University of Warwick. Her research interests lie in the areas of nonlinear and stochastic systems identification, control of system dynamics, modelling and data analysis with applications in the areas of energy and biomedical systems. Current biomedical engineering research projects include the application and development of machine learning algorithms for personalised predictive modelling and the control of blood glucose variations in people with Diabetes Mellitus, risk factor investigation in incompatible kidney transplantation and data-driven modelling in hard tissue engineering.
Invited speakers:

Prof Alexandre Loupy MD PhD

Dr Alexandre Loupy is a 36-year-old. He started his fellowship in November 2011 and is now part of the department as an associate professor at Necker Hospital. He defended his M.D in 2008 and obtained the Resident Award of Paris Hospitals. He defended his PhD in basic science in 2011 and obtained a PhD in biostatistics in 2014.

His research interest focuses on antibody mediated rejection of kidney allografts and the relationship with graft histological lesions, immune mediated vascular aging and allograft outcome. Dr Loupy has recently developed a populational approach of transplantation with new methodological tools that are used for risk stratification. This new populational approach of extremely well phenotyped cohorts has been extended to hearts, lung transplants Dr Loupy is involved in the French Society of Transplantation and the Banff Conference Scientific Committee. He has also built strong international collaborations with worldwide reference centers including the Alberta Transplant Applied Genomic Center, Edmonton, Canada, The University of Pittsburgh, USA and the Cedars Sinai Comprehensive Transplant Center, Los Angeles, USA.

Babak J. Orandi MD PhD MSc

Dr Babak J. Orandi joined the Epidemiology Research Group for Organ Transplantation in the Division of Transplant Surgery. Under the direction of Drs. Dorry Segev and Robert Montgomery, he completed a PhD in Clinical Investigation at the Johns Hopkins Bloomberg School of Public Health focused on HLA-incompatible kidney transplantation. He completed a B.A. in Spanish, a Masters in Clinical Research, and his M.D., all from the University of Michigan. In his free time, he enjoys running, traveling, reading, and yoga.

Dr Dermot Mallon, BSc MB ChB MSc

Dr Dermot Mallon is interested in the analysis of the structure and electrostatic potential of HLA B-cell epitopes; I am investigating how these relate to HLA immunogenicity and the development of humoral alloresponses.

RCS Research Fellow
Department of Surgery at the University of Cambridge. UK
Dr Rachael Coates MB ChB, BSc, MRCS (Ed) PG Cert (Transplantation)

Dr Coates’s graduated from Liverpool in 2007 and is a trainee in surgical specialties. Her research interest lies in second use kidney such as tumour excised kidneys for transplantation.

Professor Richard J Pleass, BSc, MSc, PhD

Richard Pleass graduated in Zoology from King’s College London in 1990. After an MSc in Medical Parasitology from the London School of Hygiene and Tropical Medicine he obtained his PhD in Parasite Immunology from Imperial College London in 1994. He moved to the University of Dundee to work on IgA, and as a Wellcome Trust Advanced Training Fellow developed recombinant human antibodies targeting the malaria parasite. He became a lecturer at the Institute of Genetics, University of Nottingham in 2003. With 5 yr MRC and EU career development awards he became Associate Professor in 2007 before joining the Liverpool School of Tropical Medicine in 2010.

Research
The structural diversity of the Fc receptor family and their broad distribution on different cells of the immune system enables them to mediate a plethora of biological functions as diverse as antigen presentation, phagocytosis, cytotoxicity, induction of inflammatory cascades and modulation of immune responses. Parasites, in order to survive in the immune competent host, have devised ingenious methods to subvert this important aspect of the immune response. Using a number of approaches, including human FcR transgenic and knockout models, we are dissecting the roles of FcR receptors in immunity to parasites and utilizing this information to drive forward the development of novel self-adjuvantizing vaccines for the neglected tropical diseases. My laboratory is currently investigating why erythrocytes infected with malaria parasites bind the Fc region of IgM.

Professor Kathryn Wood

Kathryn Wood runs the Transplantation Research Immunology Group (TRIG – www.nds.ox.ac.uk/trig). Her research focuses on tolerance induction at the molecular and cellular level, immune regulation and interactions between the immune system and stem cell derived tissues. Professor Wood is a Fellow of The Academy of Medical Sciences and her research achievements have been recognised internationally, including receiving a Gold Medal awarded by The Catalan Society of Transplantation (2011), The Maharshi Sushruta Award (2012) and the TTS Women in Transplantation, Achievement Award (2014). Kathryn was President of The Transplantation Society (2004-2006); was the founding Chair of the Women in Transplantation initiative (WIT – www.tts.org/women) and Editor of Transplantation (1992 - 2014).
Professor Anthony N Warrens DM PhD FRCP FRCPath FEBS FHEA

Prof Anthony Warrens is currently the president of the British Transplantation Society. He is the Dean for Education, Barts and The London School of Medicine and Dentistry, Queen Mary, University of London.

Clinical interests: general nephrology, transplantation medicine, immunology
Research interests: attitudes to organ donation; mechanisms of rejection, significance of anti-HLA antibodies

Prof James Neuberger

After qualifying in medicine in Oxford, I did junior medical training in London and Leeds before moving to the Liver Unit at Kings College Hospital where I was a research fellow and later Hon Senior Lecturer. I moved to Birmingham in 1978 and remained a Consultant there; in 2007, I was appointed Associate medical Director, ODT. I have published extensively in liver disease and transplantation; am currently an editor of the journal Transplantation, on the boards of several journals

Dr Paul Harden FRCP

He trained for six years as a nephrologist and transplant physician at the Western Infirmary in Glasgow. Subsequently he worked as a Consultant Nephrologist with a special interest in transplantation at the University Hospital of North Staffordshire. During this period he developed an interest in non-adherence in young adult transplant recipients and transition of care from paediatric to adult care. He established a joint transition process with Birmingham Children’s Hospital. In 2002, Dr Harden joined the Oxford Kidney Unit and Transplant Centre and has continued to pursue his interest in transition, having established joint clinics with Great Ormond Street and Evelina Children’s Hospitals in London. He is clinical advisor to the National Health Service in the UK on adolescent transition and young adult services in patients with ESRD. He runs a unique community-based young adult transplant service in Oxford. In addition Paul Harden is interested in malignancy post-transplantation and the impact of targeted immunosuppression reduction. He is Chief Investigator of the RESCUE (UK) trial of immunosuppression modulation for squamous cell skin cancer post-transplantation. He is currently working with a European consortium on development of a trial of cell therapy to allow immunosuppression reduction
Prof Derek Middleton PhD FRCPATH D.Sc

His clinically and research interests revolve around Histocompatibility and Immunogenetics. He has been involved in 350 publications dealing with HLA and KIR genes in transplantation, disease studies and methodologies. Currently he is heavily involved in determining different ways of trying to obtain a successful transplant for immunologically difficult patients and in the website www.allelefrequencies.net

Head of H&I laboratory at Liverpool, UK

Dr. Robert Vaughan PhD FRCPATH

Dr Vaughan's laboratory facilitate over 350 renal transplants per annum including all the paediatric renal transplants in the London area. We regard HLAi transplants to refer only to transplants that require physical antibody removal. With clinical colleagues we have developed a programme to gauge the level of HLA specific antibody to donors and predict the number of removal procedures required to allow successful transplantation. This rational approach to HLAi has been applied to renal and stems cell transplantation and is being extend to include both donors from the LD sharing scheme and deceased donors for highly sensitised long-waiters.

Director of Clinical Transplantation Laboratory at Guy's & St Thomas' Foundation Trust & King's College, London.

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