Author/s:
Clark, B;Carter, C;Wilks, DJ;Lobb, M;Hughes, P;Baker, R;Kay, SPJ

Title:
The Leeds hand transplant programme: Review of the laboratory management of the first six cases

Date:
2020-02-01

Citation:
Clark, B., Carter, C., Wilks, D. J., Lobb, M., Hughes, P., Baker, R. & Kay, S. P. J. (2020). The Leeds hand transplant programme: Review of the laboratory management of the first six cases. International Journal of Immunogenetics, 47 (1), pp.28-33. https://doi.org/10.1111/iji.12466.

DR BRENDAN CLARKE (Orcid ID : 0000-0001-7243-1916)

Article type : Original Manuscript

The Leeds Hand Transplant Programme: Review of the Laboratory Management of the First Six Cases

Brendan Clark[1], Clive Carter[1], Daniel J. Wilks[2], Mark Lobb[1], Pamela Hughes[1], Richard Baker[3] and Simon P.J. Kay[2].

Departments of [1]Transplant Immunology, [2]Plastic and Reconstructive Surgery [3] Renal Medicine, Leeds Teaching Hospitals, Leeds, UK.

Corresponding author: Brendan Clark, Transplant Immunology, Level 09, Gledhow Wing, St James's University Hospital, Leeds, LS97TF. Email: brendanclarke@nhs.net. Tel: +00441132066490

Abstract

The UK hand transplantation programme is hosted by the Department of Plastic and Reconstructive Surgery at Leeds Teaching Hospitals under the leadership of Professor Simon Kay. Since programme launch in 2013 ten procedures in six individuals have been performed involving unilateral or bilateral transplants. The multi-disciplinary team that delivers the programme includes the Transplant

Immunology service. The laboratory experience in programme support is reported here.

Keywords: HLA, antibody, transplant, hand, post-transplant, immunophenotype

Introduction

Hand transplantation (HT) is a novel clinical intervention allowing restoration of function and appearance to patients who have lost limbs to illness or trauma. Worldwide experience now exceeds one hundred cases including unilateral and bilateral procedures (Park, 2019). In distinction to solid organ transplantation (SOT) HT is considered to be a life-enhancing rather than life-saving surgery which involves a composite tissue graft. Complexity is therefore added into the clinical risk vs benefit evaluation and post-transplant patient management. The risks attending the necessary life-long immunosuppression present an ethical dilemma in this context.

Patients presenting for HT represent a range of challenges including complex surgical, clinical, psychological, social and immunological factors. This requires involvement of a large multidisciplinary team to determine candidacy and approach. Relatively few centres have the capability to assemble the required body of expertise for successful programme delivery.

Clinical outcomes, especially in programmes where rigorous patient selection is in place and good follow-up maintained, have been encouraging with restored ability to perform routine daily activities as well as the other complex functions of the hand. Patients identify an improved quality of life following transplant (Petruzzo, 2010)

As clinical experience has developed the role of Human Leucocyte Antigens (HLA) in determining clinical outcome, both with respect to matching and recipient sensitisation has begun to emerge (Bonastre, 2012). This relationship is not unexpected since a considerable literature exists that identifies an influence of HLA matching on skin allograft survival (Jonker,1979*),* of HLA expression by peripheral

nerve cells (Meyer zu Horste, 2010) and of sensitisation against donor HLA following vascular allografting (Watson, 2016).

A peculiar restriction to HLA matching in HT however exists in that the recipient and available donor pools are both small. This is compounded by the need to achieve a physical size and appearance match which usually necessitates skin tone age and sex concordance.

The Leeds programme was initiated in 2012 and has now successfully performed ten transplants in six recipients, making it one of the most active centres in the world. These include the first transplant to be performed synchronously with limb amputation

The achievement reflects the joint efforts of a wide multi-disciplinary team (MDT) including surgeons, physicians, therapists, nursing staff and laboratory scientists.

This paper presents the case experience to date with respect to laboratory findings and clinical outcomes.

Methods

Pre-transplant workup

Following identification as a candidate for transplantation each patient is HLA-A,B,C,DR,DQ typed by a PCR-Sequence Specific Oligonucleotide Probe (SSOP) method (LABType, One Lambda) and screened for HLA antibody (LABScreen Mixed, One Lambda) with determination of specificity in positive cases (LABScreen Single Antigen, One Lambda). HLA typing is repeated on a second sample by an in-house PCR-Sequence Specific Primers (SSP) method and screening on a three monthly cycle in accordance with UK guidelines for solid organ transplantation. Results of single antigen bead analysis are interpreted as positive for specificities with median fluorescence intensity (MFI) >2000. Based on these results the clinical team are provided with the patient calculated reaction frequency (CRF) to enable discussion with the patient regarding their chance of a compatible donor being found.

Antibody defined unacceptable antigens are accumulated into the patient record longitudinally, with a minimum of four screens performed before activation of the patient onto the wait-list.

Activation

Following MDT discussion patients are activated to receive donor offers. Following activation HLA antibody screening with specificity analysis as indicated is performed monthly. Detected HLA antibodies constitute a veto to use of donors with cognate antigen and are recorded into the patient laboratory record for review against offered donors.

Time of offer

This process is co-ordinated between the Leeds surgical and local NHS-Blood and Transplant (NHSBT) Specialist Nurse in Organ Donation (SNOD) teams with laboratory involvement following initial donor assessment by surgeons. Where local donors have been found donor HLA typing will have been performed by the laboratory. In the case of donors from elsewhere donor HLA typing results are requested from the national transplant database held by NHSBT.

As a national programme recipients are distributed across the UK and need to be transported to Leeds to receive their transplant. To date in the UK programme, owing to logistical and clinical considerations, only donation after brainstem death (DBD) donors have been accepted as the source of upper limbs for transplant. Initially a geographic restriction operated limiting retrieval just to donors within hospitals in the West Yorkshire area but this has now been extended across a larger area of Northern England. Because of the sensitivity surrounding disfiguring donations donor limb amputation should be avoided if immunological barriers to transplantation are present. A pressure of time however exists since amputation of donor limbs occurs as the first event in the retrieval process. Accordingly assessment of recipient-donor

compatibility needs to be performed within imposed time-constraints. This also avoids unnecessary disturbance of the recipient. This has been achieved using either a donor peripheral blood (PBL) crossmatch against recipient current sample or virtual crossmatch approach.

Post-transplant

After transplant patients are closely monitored for development of allosensitisation and graft rejection. This includes screening for *de-novo* appearance of donor HLA specific antibody, [+3, 7, 14, 28 days, monthly for the next two months and then quarterly thereafter unless indicated for clinical reason] lymphocyte subset analysis and for-cause biopsy. Lymphocyte subset analysis was performed at the same timepoints using Trucount tubes and appropriate antibody panels by flow cytometry to measure percentage and absolute numbers of B, T and NK cells as previously described (Arumgakani 2010).

Results

Table 1 presents laboratory data and clinical outcomes.

The programme presently aims to deliver two to three transplants per year. An active wait-list of usually no more than four patients is maintained. Since 2012 a total of 46 patients have had their laboratory work-up for transplantation completed. This balance reflects the time and efforts taken in ensuring both the physical, psychological, medical and surgical suitability of candidate patients. Patients that do not progress to transplant account for more than half of the total.

DBD donors have been used exclusively as the source of limbs This situation relates to the more predictable process flows around such donors which enables forward planning for the transplant and physiologic considerations relating to perfusion of the peripheries which are more significant in situations resulting in cardiac death . Donors have been recruited across a limited geographic area to allow on-site surgical review and to limit ischaemia time. If the decision is made to proceed limbs are retrieved prior to solid organs if clinical conditions allow.

Match grades have been dictated by donor surgical suitability and limb appearance requirements of the recipient.

All transplants have been performed avoiding antibody incompatibilities and following a negative prospective or virtual crossmatch based on a current sample. Absence of donor HLA specific antibody was retrospectively confirmed in all time-of-offer samples

Four of six patients had lymphocyte subset analysis to measure the percentage and absolute numbers of B, T and NK cells prior to transplantation. This showed that all lymphocyte populations were within the normal adult range prior to transplantation (data not presented).

All patients received alemtuzumab perioperatively. This resulted in the complete removal of peripheral blood lymphocytes in all six cases . Following surgery and under cover of the identified immunosuppressive drug regimens, lymphocytes slowly repopulated, with NK cells the first to be detected (in all six patients). Whilst there was evidence of limited T cell repopulation in all patients, only one patient (patient 1) had T cell numbers within the normal adult range. Significant numbers of B cells could be detected in only one of the six patients. Table 1 provides data from the most recent sample in each case.

Where T cell repopulation had occurred, further flow analysis revealed that repopulating T cells initially had predominantly memory phenotype ($CD3^+$ $CD4^+$ $CD45RA^-$ $CD45RO^+$). In patient one, assayed at one year post transplant, 94% of $CD4^+$ T cells were memory ($CD45^{RO+)}$ with only 3 % naïve ($CD45^{RA+}$). The remaining 3% of T-helper cells expressed both $CD45^{RA}$ and $CD45^{RO}$. By five years post-transplant, naive T cells had increased to 15% with a concomitant reduction in memory T cells (79%). 6% of $CD4^+$ T-cells expressed both $CD45^{RA}$ and $CD45^{RO}$ (data not presented)

Memory B cell phenotype was additionally tested in patients where lymphocyte marker analysis indicated B cell repopulation was underway. In patient one, tested at one year, 91% of peripheral blood B cells were naïve ($CD27^-$) with only 5% $CD27^+$

memory B cells (4% of B cells were $CD27^-$ IgD^-). By five years post-transplant, the proportion of $CD27^-$ naïve B cells had reduced to 78% of total B cells, with memory B cells increased to 18% (7% (IgD^+) non switched and 11% switched (IgD^-) memory B cells) (data not presented)

All grafts remain *in-situ* with good function which is still improving in later cases.

Discussion

We present a series of ten hand transplants (two unilateral, four bilateral) in six individuals representing the total UK experience to date.

From numbers involved it can be seen that the laboratory completes approximately eight work-ups to achieve one transplant. This reflects a stringent patient selection process which identifies candidates for whom best clinical outcomes can be predicted and that demonstrate the necessary resilience and fortitude to cope with the physical and psychological demands. The process also results in a number of patients de-selecting themselves as they evaluate and balance their personal gains and risks.

In common with other series limitations on available donors, with respect to recipient cosmetic requirements, surgical considerations and consent, has meant that we have been unable to direct donors to recipients on the basis of HLA matching. In practice the allocation is made clinically and notified to the laboratory at point of request for assessment of compatibility. Where a pairing is identified as incompatible on the basis of recipient immunology reallocation does not occur and the limbs are not retrieved. Higher levels of mismatching are therefore a feature of our cohort with an average of five mismatches across HLA-A, B, DR loci. A recently published multicentre study (Berglund, 2019) has identified a correlation between HLA-CII mismatching and post-transplant appearance of donor HLA specific antibody, which in turn correlated with the incidence of acute cellular rejection. Cellular rejections

preceded antibody development in almost all cases. With the exception of our most recent case, and in common with the reported experience of others (Petruzzo,2010) we have recorded multiple, histologically proven, cellular acute rejection episodes in all of our cases. These have been classified as moderate to severe in accord with Banff criteria (Cendales, 2008). All have been reversible with increased immunosuppression.

To date however, and with up to six years of follow-up, we have not seen the *de-novo* appearance of donor-HLA specific antibody in any of these. This situation may reflect the immunosuppression employed which includes intra-operative alemtuzumab and a triple maintenance regime of tacrolimus, prednisolone and mycophenolate mofetil.

In our programme to date we have maintained a precautionary approach to pre-existing donor HLA-specific sensitisation and have thus far vetoed transplantation in the presence of donor antibody. This has had implications in terms of wait-time to offer for certain patients for whom multiple prospective donors have been identified but then declined on this basis. Consideration has been given to HLA incompatible (HLAi) transplantation in the context of one such patient. This need was however avoided when a donor without antibody conflicts was eventually found. Given the clinical background of patients in the programme it is however inevitable that this situation will again present and a HLAi route to transplantation will need to be considered. In this regard the centre has an established antibody incompatible renal transplant programme and ample transferrable experience of patient management in this setting. The reported experience of transplant of composite tissue across antibody boundaries is however scant, being limited to two case reports concerning the management of antibody mediated rejection in pre-sensitised recipients of full-face allotransplants (Chandraker, 2014; Chandraker 2016). Both cases required considerable efforts to reverse and exposed the recipients to high levels of a number of potent immunosuppressive modalities. Whether use of such a heavy immunosuppressive regime can be justified in the setting of hand transplantation is debatable.

The logistics of limb retrieval and desire to ensure as short a cold ischaemia time as possible has dictated our approach to crossmatch. Initially a donor PBL crossmatch was employed but was replaced from the second case onwards by a virtual crossmatch approach facilitated through a locally maintained database of antibody defined unacceptable antigens. This has allowed a prompt assessment of compatibility and helped minimise impacts of the programme on donor management with respect to other organs for transplant.

Post-transplant, lymphocyte repopulation occurred in line with previous observations of patients following alemtuzumab treatment (Cherukuri, 2012). NK cells were the first lymphocyte population to be observed with absolute numbers within normal range in 6/6 patients. T cell repopulation has occurred in the first patient to be transplanted with the absolute numbers of CD4 and CD8 T cells within adult normal range at 5 years post-transplant. However 5/6 patients remain T cell lymphopenic (in both CD4 and CD8 compartments). Notably, two of these patients are now 3 years post-transplant. There was no obvious relationship between lymphocyte repopulation and rejection timepoints, although the significance of this finding is unclear given the small number of patients and the relatively short length of time since transplant .
Where T cells were detected, they were predominantly of a memory phenotype, with the majority expressing $CD45^{RO}$ and lacking expression of $CD45^{RA}$. This profile is consistent with that seen following alemtuzumab treatment in other clinical scenarios, such as following renal transplantation or in the treatment of multiple sclerosis (Cherukuri 2012, Hill-Cawthorne 2012). 5/6 patients were profoundly B cell lymphopenic, with only the first patient with B cells in the normal adult range (168 cells/μl) at the time of last assessment. In this case B cells had initially repopulated with a cell number of 539 cells/μl being achieved before declining to current levels. B cell repopulation was very slow in 5/6 patients, which may be related to the level of immunosuppression being administered. Where B cells have repopulated, it has proceeded in a manner consistent with other reports concerning use of therapeutic B cell depleting agents (Becerra 2014, Avanzini 2005, Leandro 2006, Zaja 2007) with a dominance of naïve ($CD27^-$) B cells initially, and memory B cells slowly increasing over time. In aggregate these findings identify a cohort with phenotypic features that imply a deranged immunity with clinical implication for development of autoimmunity, infections and malignancy. All patients are closely managed for this risk.

We continue to build our experience in this rapidly developing area of transplant practice.

References

Arumugakani, G; Wood, P.M.D; Carter, C.R.D (2010) Frequency of Treg cells is reduced in CVID patients with autoimmunity and splenomegaly and is associated with expanded CD21^{lo} B lymphocytes. J Clin Immunol, 30, 292-300.

Avanzini, M.A; Locatelli, F; Dos Santos, C; Maccario, R; Lenta, E et al. (2005). B lymphocyte reconstitution after hematopoetic stem cell transplantation: functional immaturity and slow recovery of memory CD27+ B cells. Exp Hematol. 33, 480-486

Becerra, E; Scully, M.A; Leandro, M.J; Heelas, E.O, Westwood, J.P et al. (2014). Effect of Rituximab on B cell phenotype and serum, B cell-activating factor levels in patients with thrombotic thrombocytopenia purpura. Clin and Exp Immunol 179, 414-425

Berglund, E; Andersen Ljungdahl, M; Bogdanović, D; Berglund, D.et al (2019) Clinical significance of alloantibodies in hand transplantation - a multicentre study. Transplantation. 22. doi: 10.1097/TP.0000000000002650. [Epub ahead of print]

Bonastre, J; Landin, L; Jesus, D; Casado-Sanchez, C; Casado-Perez, C (2012) Factors Influencing Acute Rejection of Human Hand Allografts.A Systematic Review. Annals of Plastic Surgery, 68, 624–629.doi: 10.1097/SAP.0b013e318255a411

Cendales, L.C; Kanitakis, J; Schneebeger, S; Burns, C; Ruiz, P et al. (2008) The Banff 2007 working classification of skin-containing composite tissue allografts. Am. J. Transplant, 8, 1396-1400 doi: 10.1111/j.1600-6143.2008.02243.x

Chandraker, A; Arscott, R; Murphy, G.F; Lian, C.G; Bueno, E.M et al. (2014) The management of antibody-mediated rejection in the first presensitized recipient of a full-face allotransplant. Am J Transplant,14,1446-1152. doi: 10.1111/ajt.12715.

Chandraker, A; Arscott, R; Murphy, G; Lian, C; Bueno, E et al (2016) Face Transplantation in a Highly Sensitized Recipient. Mil Med 181(5 Suppl):221-6. doi: 10.7205/milmed-d-15-00136.

Cherukuri, A; Salama, A.D;Carter, C; Smalle, N; McCurtin, R et al, (2012). An analysis of lymphocyte phenotype after steroid avoidance with either alemtuzumab or basiliximab induction in renal transplantation. Am. J. Transplant, 12: 919-931

Hill-Cawthorne, G.A; Button, T; Tuohy, O; Jones, J.L; May, K et al (2012) Long term lymphocyte reconstitution after alemtuzumab treatment of multiple sclerosis. J Neurol. Neurosurg. Psychiatry 83, 298-304

Jonker, M; Hoogeboom, J; van Leeuwen, A; Koch, C.T; Blusse van Oud Alblas, D et al (1979) Influence of matching for HLA-DR antigens on skin graft survival. Transplantation, 27, 91-4

Leandro, M.J; Cambridge, G; Ehrenstein, M.R; Edwards, J.C.W (2006). Reconstitution of peripheral blood B Cells after depletion with Rituximab in patients with rheumatoid arthritis. Arthritis and Rheumatology 54, 613-620.

Meyer zu Horste, G; Holger, H; Lehmann, H.C; Ferrone, S; Hartung, H-P et al; (2010) Expression of antigen processing and presenting molecules by Schwann cells in inflammatory neuropathies. Glia. 58, 80-92. doi:10.1002/glia.20903

Park, S.H; Eun, S.C; Kwon, S.T (2019) Hand Transplantation: Current Status and Immunologic Obstacles. Exp Clin Transplant, 17, 97-104. doi: 10.6002/ect.2018.0163.

Petruzzo, P; Lanzetta, M; Dubernard, J.M; Landin, L; Cavadas, P et al (2010) The International Registry on Hand and Composite Tissue Transplantation. Transplantation, 90, 1590-4. doi: 10.1097/TP.0b013e3181ff1472.

Watson, H; Pande, R; Farid, S; Ecuyer, C, Baker, R et al (2016) Non-HLA-matched 3rd party vascular allograft in renal transplant may lead to sensitization against donor HLA. Clin Transplant,30,1508-1512. doi: 10.1111/ctr.12831.

Zaja, F; Bacigalupo, A; Patriarca, F; Stanzani, M; van Lint, M.T et al (2007). Treatment of refractory chronic GVHD with Rituximab: a GITMO STUDY. Bone Marrow Transplantation, 40: 273-277

Table 1. Laboratory data and clinical outcomes for recipients of hand transplants in the UK programme.

Patient #	1	2	3	4	5	6
CRF (time of transplant)	0%	0%	0%	0%	0%	74%
Crossmatch	Donor PBL	Virtual	Virtual	Virtual	Virtual	Virtual
Number of donors assessed for compatibility	1	1	2	1	1	5
Date of Tx	26/12/2012	16/07/2016	02/03/2017	12/09/2018	17/09/2018	08/01/2019
Uni/bilateral	Unilateral	Bilateral	Bilateral	Bilateral	Unilateral	Bilateral
HLA mismatch (A,B,DR)	112	122	212	021	222	222
Donor HLA	A*24, A*31; B*40, B*44; C*03, C*07; DRB1*04, DRB1*08; DQB1*03, DQB1*04;	A*02, A*24; B*40, B*49; C*03, C*07; DRB1*03, DRB1*13; DQB1*02, DQB1*06;	A*01, A*03; B*07, B*08; C*07; DRB1*03, DRB1*15; DQB1*02, DQB1*06;	A*01, A*02; B*40, B*49; C*03, C*07; DRB1*01, DRB1*13; DQB1*05, DQB1*06;	A*02, A*24; B*15, B*51; C*03, C*15; DRB1*07, DRB1*11; DQB1*02, DQB1*03;	A*01, A*26; B*14, B*49; C*07, C*08; DRB1*03, DRB1*13; DQB1*02, DQB1*06;

	DPB1*03, DPB1*04	DPB1*04	DPB1*06, DPB1*14	DPB1*02, DPB1*04	DPB1*02, DPB1*11	DPB1*02
Recipient HLA	A*01, A*23; B*08, B*44; C*07, C*14; DRB1*07, DRB1*13; DQB1*02, DQB1*06	A*02; B*07, B*57; C*06, C*07; DRB1*12, DRB1*15; DQB1*06, DQB1*07	A*02, A*31; B*07, B*56; C*01, C*07; DRB1*01, DRB1*11; DQB1*03, DQB1*05	A*01, A*02; B*08, B*27; C*0(1, C*07; DRB1*01:03, DRB1*03; DQB1*02, DQB1*03	A*03, A*32; B*07, B*39; C*07; DRB1*04; DQB1*03	A*11, A*24; B*15, B*51; C*03, C*15; DRB1*01, DRB1*11; DQB1*03, DQB1*05
Induction therapy	Pred 1000mg Alemtuzumab 20mg	Pred 1000mg Alemtuzumab 30mg	Pred 1000mg Alemtuzumab 30mg	Pred 1000mg Alemtuzumab 30mg	Pred 1000mg Alemtuzumab 30mg	Pred 1000mg Alemtuzumab 30mg
MaintenanceTherapy	Pred Tac MMF	Pred Tac MMF	Pred Tac MMF	Pred Tac MMF	Pred Tac (with switch to Ciclosporin) MMF	Pred Tac MMF
AR #	13	4	3	5	2	0
Day of AR episodes (Banff score)	Day 51 (Grade 3) Day 97 (Grade 2) Day 149 (Grade 3) Day 163 (Grade 2)	Day 59 (Grade 2) Day 146 (Grade 2)	Day 56 (Grade 1) Day 75 (Grade 2)	Day 57 (Grade 2) Day 92 (Grade 2)	Day 122 (Grade 2) Day 186 (Grade 2)	Nil to date

	Day 198 (Grade 3) Day 756 (Grade 3) Day 888 (Grade 2) Day 917 (Grade 2) Day 1153 (Grade 3) Day 1299 (Grade X) Day 1317 (Grade 3) Day 1721 (Grade X) Day 2256 (Grade 3)	Day 198 (Grade 2) Day 230 (Grade 2)	Day 678 (Grade 2)	Day 127 (Grade 2) Day 136 (Grade 3) Day 191 (Grade 2)		
Graft loss	No	No	No	No	No	No
Serum DSA before Tx (HLA class & MFI)	Nil	Nil	Nil	Nil	Nil	Nil
Serum DSA post-Tx (HLA class, MFI & date for each positive detection)	Nil	Nil	Nil	Nil	Nil	Nil
Lymphocyte subset absolute(cells/μl)	CD3: 727 CD4: 314 CD8: 267	CD3: 107 CD4: 67 CD8: 31	CD3: 105 CD4: 74 CD8: 23	CD3: 275 CD4: 146 CD8: 160	CD3: 110 CD4: 57 CD8: 49	CD3: 116 CD4: 10 CD8: 91

in most recent sample (Months post-transplant)	NK: 248 B cell: 89 (80)	NK: 91 B cell: 9 (36)	NK: 118 B cell: 44 (27)	NK: 234 B cell 1 (12)	NK: 87 B cell: 11 (9)	NK: 105 B cell: 5 (6)